HUMORAL INFLUENCE OF THE HYPOTHALAMUS ON GONADOTROPHIN SECRETIONS

BY

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Date:

Approved:

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A dissertation

submitted in partial fulfillment of
the requirements for the degree
of Doctor of Philosophy
in the Graduate School
of Arts and Sciences
of
Duke University

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HISTORY AND INTRODUCTION

Although the pituitary gland has been known since the time of Galen in 200 A.D., no serious attempt was made to elucidate its structure and function until the 19th century. Hannover (1844) studied the structure and differentiated two cell types in the pars distalis. Flesch (1884) and Dostoiewsky (1884) independently resumed the structural study and differentiated chromophobe and chromophil cells. Saint-Remy (1892) postulated that the cell types simply represent different phases of secretion. Herring (1908) systematically demonstrated the various cell types of the pars distalis in different species. He also pointed out the presence of colloidal structures in the posterior lobe. He believed that a substance is secreted by the intermediate lobe and is transported to the posterior lobe by lymphatic channels and via the stalk to the third ventricle. He was impressed by the tremendous vascularization of the pars distalis and realized that its blood supply comes from branches of the internal carotids which enter the gland at the sides of the infundibulum and break up immediately into larger,

thin-walled vessels which descend along the stalk and enter the pars distalis.

Experimentation with respect to function really began early in this century. It was first sought to discover whether or not the gland is indispensable for life. Paulesco (1908) stated that dogs die within 24 hours after total hypophysectomy. Camus and Roussy (1913a, 1913b) described experiments in dogs in which they either performed hypophysectomy or made crude lesions in the hypothalamus. They observed a polyuria, which followed both the hypothalamic lesion and the subtotal removal of the pituitary. They also noted a progressive testicular atrophy in two animals with lesions.

Crowe (1909) showed that transplantation of the pituitary into a hypophysectomized dog significantly prolonged the life of the animal. Crowe, Cushing and Homans (1910) showed, in dogs, that hypophysectomy is incompatible with life and mentioned the adiposogenital syndrome which appeared in some dogs after hypophysectomy. Aschner (1912) emphasized that the injury to the hypothalamus could have been the cause of some of the results that had been obtained. Bailey and Bremer (1921) used the temporal approach to the pituitary gland in dogs. This permitted the production of hypothalamic lesions without causing any injury to the gland. The experiments are documented with a histological study of the pars distalis showing its normal structure. Three animals, treated in this manner, show the adiposogenital syndrome, and the authors suggested that all the disturbances usually ascribed to

the hypophysectomy were due really to tuberal lesions.

It was only in 1926 that the basic function of the anterior lobe was elucidated by the classical experiments of Smith, and Smith and Engle (1926, 1927, 1930). Its influence on the gonads was of particular significance. In experiments with rats, Smith found that after hypophysectomy, there was an atrophy of the genital organs in adults or lack of development of these organs in immature animals. In the adult female he found a complete arrest of cycles. In other experiments hypothalamic lesions were made and the adiposogenital syndrome resulted. The genital atrophy which followed these lesions was not as pronounced as it was after hypophysectomy, and some irregular cycling was observed. There was no observable atrophy of either the thyroid or the adrenals. The disturbance which occurred in hypophysectomized animals could be repaired by daily injections of anterior pituitary gland. Smith also showed that injection of anterior pituitary substance in immature animals causes precocious puberty, and in adult animals hypertrophy of the genital organs. In castration experiments, he showed that the pars distalis acts directly on the gonads which in turn stimulate the accessory organs.

Isolation of the "active principles" soon followed. The separation and purification of two anterior pituitary gonadotrophins, the follicle stimulating hormone (FSH) and the luteinizing hormone (LH), was accomplished by Evans and Simpson (1928), Fevold et al. (1931, 1933, 1941, 1943), Greep et al. (1940) and Shedlovsky et al. (1940). It was found that FSH acted on

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follicular development and that LH induced ovulation with subsequent formation of corpora lutea. Astwood (1941) discovered a third gonadotrophic hormone, the luteotrophin (LTH), which was later identified with lactogen by Evans, Simpson and Lyons (1941), and Evans, Simpson, Lyons and Turpeinen (1941). No other gonadotrophic hormones have, to the present time, been postulated and it is believed that FSH, LH, and LTH are the main "principles" in the control of gonadal function.

Hypophyseal-gonadal interrelationships (in the form of the "push-pull" hypothesis) were first reported by Moore (1930) and Brouha and Simonnet (1930), and extended by Moore and Price (1932). Brouha and Simonnet believed that the variation of estrogen concentration, through its influence on the pars distalis, plays an essential role in the production of estrous cycles. Moore and Price stressed the fact that the hypophyseal activity is to some extent controlled by gonadal secretion, for hypophyseal activity is lowered when the gonadal hormones are present in effective amounts.

Before this time, there had been a few reports which strongly suggested such an interrelationship between the gonads and the pars distalis. It had been noticed by Fichera (1905), in a variety of species, that the weight and structure of the pars distalis was markedly altered, from the controls, if the animals had been castrated for some time. A histological study showed a large increase in basophil cells, especially a kind containing large vacuoles (later called castration cells). The increase apparently occurred at the expense of the acidophils. These changes could

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be obviated if, after castration, the animals were given daily injections of fresh gonadal extracts. Schleidt (1913) also reported castration cells in the pars distalis after gonadectomy and showed that the changes could be obviated by transplantation of ovaries or testes into the castrated animals. Hatais (1913) and Addison (1917) gave further proof of the changes that occur in the basophils following castration.

The question of whether the influence of gonadal hormones on the pars distalis is a direct one or whether it is mediated by the central nervous system was raised by Hohlweg and Junkmann (1932). They transplanted the anterior lobe to the kidney in a group of rats and at the same time performed gonadectomies. The control group was only gonadectomized. The anterior lobes of the controls underwent definite castration changes, while no such changes were observed in the experimental group. Furthermore, when animals were injected with atropine following castration, the appearance of castration cells in the intact pituitary was prevented. The authors, on this basis, postulated a "sex center" in the hypothalamus through which the action of gonadal hormones on the pars distalis would be affected. It was shown by Desclin and Gregoire (1936), using pituitary transplants, and by Westman and Jacobsohn (1938a), working with stalk-sectioned animals with the placement of a barrier to prevent regeneration of nervous connections, that estrogen can have a direct effect on the pars distalis. This direct effect, however, although present, is limited

to the production of mitoses in the gland and does not seem to enhance its secretory activity.

If a "sex center" does exist, the mechanism by which it can influence the anterior pituitary gland is an important consideration. Anatomically, two possible connections between the hypothalamus and the pars distalis may exist: (1) by a direct nerve supply and (2) by a vascular connection. Over the years, the anatomy of the region has been studied in great detail to determine the existence of a suitable innervation, a suitable blood supply, or both. From the literature, it is seen that very few authors believe that the pars distalis receives an abundant innervation. Pines (1925), studying human pituitaries, stated that the anterior lobe has an abundant sympathetic innervation, while Vasques-Lopez (1949), studying horse pituitaries, found numerous nerve fibers leaving the hypothalamo-hypophyseal tract and innervating the anterior lobe. On the other hand, Berkley (1894), Dandy (1913) and Croll (1928) believed that the anterior lobe innervation is composed of only very few autonomic fibers. Hair (1937, 1938) and Brooks and Gersch (1941) reported that, in addition to the autonomic innervation, the pars distalis receives a few aberrent fibers from the hypothalamo-hypophyseal tract. Rasmussen (1938), Drager (1944), Green and Harris (1947) and especially Green (1951), who studied the problem in 76 species, found a complete lack of anterior lobe innervation. It is significant that these and other authors agree that an impressive number of fibers coming from the hypothalamo-hypophyseal tract end around blood vessels

in the pars tuberalis.

The existence of portal blood vessels passing between the hypothalamic region and the pars distalis was first described by Popa and Fielding (1930). These authors believed that the direction of flow in these vessels is from the pituitary towards the brain. By direct observation of such vessels in the toad, Houssay, Biasotti and Sammartino (1935) found that the direction of flow is from the brain toward the pituitary gland. This observation was documented with the finding that injury to the portal veins, at the level of the infundibulum, is followed by a necrosis of the anterior lobe. Wislocki and King (1936) also concluded that the direction of flow, in the portal vessels of the monkey, is toward the pituitary gland. Wislocki (1937, 1938) confirmed this fact in the cat and performed a more extensive study in the monkey.

Since these original findings, the work of Houssay et al., and of Wislocki and King has been confirmed a number of times. By direct observation of hypophyseal portal vessels, Green (1946) in the bull frog, Green and Harris (1949), Barrnett and Greep (1951a), and Daniel and Prichard (1954) in the rat, and Worthington (1955a) in the mouse, all agree that the direction of flow in these vessels is toward the pars distalis. The most recent studies were done by Xuereb, Prichard and Daniel (1954) in man, by Daniel and Prichard (1956) in the rat, and (1957) in sheep. These authors demonstrated very convincingly that the sole blood supply to the pars distalis comes to it through the hypophyseal portal vessels.

From these investigations, it can be seen that the pars distalis is completely deprived of arterial blood. The entire vascular supply is provided by the portal veins which bring a "special" blood that has previously circulated through the median eminence or infundibular stem, structures rich in nerve endings.

The lack of nerve fibers in the pars distalis, on the one hand, the existence of numerous nerve fibers ending around the portal vessels in the pars tuberalis and around the proximal capillary loops in the median eminence and elsewhere, on the other hand, provide anatomical grounds which suggest that the hypothalamic control of the pars distalis (if such control exists) is neurohumoral rather than neural.

Different approaches have been used to test the possible existence of a "sex center". Marshall and Verney (1936) were the first to employ electrical stimulation of the brain. In the female rabbit, a species known to be a reflex ovulator, massive electrical stimulation of the brain or spinal cord produced ovulation. Using the same species, Haterius and Derbyshire (1937), by means of very small electrodes, stimulated very discrete areas in the hypothalamus. When the electrodes were placed directly above and anterior to the optic chiasma, ovulation was produced, but did not occur if the electrodes were placed as little as 1.5 mm. away from this area. Markee, Sawyer and Hollinshead (1946) showed that the stimulation of the cervical sympathetic trunk of rabbits, or the cervical portion of the vagus nerve, did not

induce ovulation. Stimulation of the hypothalamus produced ovulation, while a direct stimulation of the pars distalis was ineffective. This result suggested to the authors the presence of a humoral link between the hypothalamus and the anterior lobe rather than a direct neural connection. The findings of Markee, Sawyer and Hollinshead were confirmed by Harris (1948). Using remote control stimulations, of low intensities, Harris found that ovulation was always produced when the tuber cinereum was stimulated for as short a period as 3 minutes, but not if the electrode was placed in the anterior pituitary, pars intermedia, or infundibular stem with stimulation for as long as 7 hours.

Another approach was an attempt to destroy specific areas of the hypothalamus and to study the effects on gonadotrophic secretion. Dey and his collaborators were the first to perform a systematic study using the ablation technique. Dey, Fisher, Berry and Ranson (1940) and Dey (1941, 1943), using guinea pigs, found that lesions that destroy the median eminence are always followed by genital atrophy and complete lack of cycles. Large bilateral lesions at the caudal end of the optic chiasma, seemed to inhibit LH secretion, as judged by the constant estrus which ensued. Unless they disrupted the connection with the hypothalamus, direct partial lesions of the pars distalis had no effect on gonadotrophic secretion. Hillarp (1949) using the rat, found that large lesions placed immediately anterior and ventral to the paraventricular nuclei, or small bilaterally symmetrical lesions placed caudal to the paraventricular nuclei, produced

the same ovarian changes reported by Dey et al. in the guinea pig. Hillarp interpreted this to indicate the presence, in the anterior hypothalamus, of an LH secretion center from which a well demarcated fiber system runs superficially on both sides of the median eminence toward the hypophyseal stalk. Greer (1953) showed that in similarly induced constant-estrous rats, daily injections of progesterone were followed by resumption of cycles, which persisted in 50 per cent of animals after the interruption of progesterone treatment. This shows that despite the lesion, the primary cyclic mechanism can be reactivated.

With the development of appropriate pharmacological agents, another valuable technique was available for the study of the hypothalamo-hypophyseal interrelationship. Markee, Sawyer and Hollinshead (1947) and Sawyer, Markee and Townsend (1949) using epinephrine and acetylcholine, on the one hand, and dibenamine and atropine, on the other, obtained results which suggested the existence of two components in the copulation-initiated neurogenic stimulus. The first one, sensitive to atropine, was judged to precede an "adrenergic" neurohumoral link, subject in turn to dibenamine blockade. Christian (1956) demonstrated that direct stimulation of the preoptic area induces ovulation in atropine-blocked but not in dibenamine-blocked rabbits. However, the exact site of action of the two drugs has not yet been established.

In the rat, Everett, Sawyer and Markee (1949) and Everett and Sawyer (1949a, 1949b, 1950, 1953) demonstrated that

administration of dibenamine, atropine or nembutal can prevent ovulation when injected before 2 P.M. on the day of proestrus. This pointed to the existence of a "spontaneous" neurogenic process, which causes release of the ovulatory hormone from the pars distalis. Sawyer, Everett and Markee (1949) and Everett and Sawyer (1949b) again using dibenamine and atropine as blocking agents, showed that the effect of estrogen or progesterone in promoting ovulation is mediated through the nervous system.

If the pars distalis is capable of normal function independent of hypothalamic control, then stalk-sectioning or complete removal of the gland to another site should not interfere with its normal performance, provided that good circulation be established and that no major loss of parenchyma occur.

Harris (1950) discovered, in rats, that after simple stalk-section, a regeneration of vascular connections between the hypothalamus and the pars distalis usually occurs promptly and that the pars distalis, sooner or later, resumes a normal, or nearly normal, function. Only when precaution is taken to place a barrier between the cut ends of the stalk, and thus prevent any regeneration of portal veins, is it observed that the pars distalis secretes minimally. This latter procedure is always followed by complete gonadal atrophy.

In the past, many contradictory results had been reported as to the effect of stalk-sectioning. Although a number of authors state that the pars distalis is capable of normal function following this procedure, others report opposite findings. The studies

of Harris offer an explanation for these contradictions, as he pointed out. It is most probable that the normal pituitary function following stalk-sectioning reported by some authors was due to re-establishment of vascular connections between the pituitary and the hypothalamus. Thus, Richter (1936), Dempsey (1939), Dempsey and Uotila (1940) and Uotila (1939, 1940) believed that normal gonadotrophin function can occur after stalk-sectioning. Brooks (1938), Leininger and Ranson (1943), Brolin (1945) and Greep and Barrnett (1951) and Barrnett and Greep (1951b) reported serious disturbance but not complete disappearance of gonadal function. On the other hand, Mahoney and Sheehan (1936), Hinsey (1937), Harris (1937), Westman and Jacobsohn (1937) and Westman, Jacobsohn and Hillarp (1943) noted complete gonadal atrophy following stalk-sectioning, Benoit and Assenmacher (1952) and Assenmacher and Benoit (1953a, 1953b) reported gonadal atrophy upon effective interruption of portal vessels or a high transection of the hypothalamo-hypophyseal tract in ducks (in this species, these two structures can be sectioned independently). Sectioning of nerve tracts below the region of the median eminence produced no effect on gonadal function. Westman and Jacobsohn (1938b), reported that pseudopregnancy can be produced in stalk-sectioned rats by electrical stimulation of the cervix, applied a few hours following the operation. However, no controls were included to show whether pseudopregnancy can occur after stalk-sectioning without any cervical stimulation.

As the regeneration of portal vessels obscured the results

of stalk-sectioning, so did the persistence of remnants in the hypophyseal capsule obscure the results of transplantation experiments. Many authors have stated that pituitary grafts do not secrete gonadotrophins when transplanted to sites remote from the hypothalamus. Martin (1936), Richter and Eckert (1937), Cheng and Sayers (1949), Fortier and Selye (1949) and Fortier (1951) observed complete gonadal atrophy in animals bearing anterior pituitary grafts in the anterior chamber of the eye. Westman and Jacobsohn (1940) obtained similar results and did not believe that this loss of function could be attributed to the decrease in amount of transplanted tissue. The size of the grafts, in their experiments, was sometimes 100 per cent larger than the normal gland. May (1935, 1937), Hill and Gardner (1936) and Cutuly (1941) using rats, reported normal gonadotrophin secretion from pituitary grafts transplanted to the anterior chamber of the eye or to the testes. Schweizer, Charipper and Haterius (1937) using guinea pigs, reported that eye transplants of pituitary tissue secreted only FSH, as indicated by a great follicular development in the ovaries of these animals. While the results of May and of Hill and Gardner can be explained by the fact of retention of remnants in the hypophyseal capsule, it is difficult to explain the results of Cutuly and of Schweizer and her co-workers who determined, by histological studies, the absence of any remnants.

An early experiment conducted by Greep (1936) indicated that the proximity of the hypothalamus has a very favorable effect

on the secretion of pituitary grafts. This author transplanted either newborn or adult pituitaries into the newly emptied "sellae" of adult hypophysectomized male or female rats. In a large number of cases, normal gonadotrophic function was observed, including pregnancy, delivery and lactation. A serious drawback to this experiment was the fact that the hypophyseal capsule could not be checked for remnants since it contained the graft itself. Therefore, it was impossible to state with certainty whether the gonadotrophic effects were due to the transplant or to the residual anterior lobe tissue. Harris and Jacobsohn (1952) repeated Greep's experiment and placed pituitary grafts under the median eminence by a trans-temporal approach. Since they placed the graft above the hypophyseal capsule, it was possible to determine at a later date, the completeness of the hypophysectomy. In control animals, the pituitary grafts were placed under the temporal lobe of the brain. In nearly all animals bearing pituitary grafts under the median eminence, resumption of gonadotrophin secretion was observed. In 50 per cent of the animals, mating was followed by pregnancy and delivery. No gonadotrophin secretion occurred in any of the control animals.

Thus, the usual experience has been that a complete lack of FSH and LH secretion comes about when the pituitary gland is transplanted at a distance from the hypothalamus. On the other hand, it has been demonstrated that the gland is able to secrete luteotrophin under such conditions. Using rats, Desclin (1950) found that homografts of pars distalis in the kidney were capable of secreting luteotrophin when a large quantity of stilbestrol

was implanted subcutaneously on the day before the operation. Large, functional corpora lutea were found in these animals. To determine whether the estrogen was a necessary stimulus for luteotrophin secretion, Everett (1954) performed autotransplantations of anterior pituitaries to the kidney on the day of estrus, omitting estrogen administration. He found that, without any special stimulus, luteotrophin was secreted, as shown during the following week by the decidual reaction to uterine traumatization. These experiments were of short duration and the possibility existed that the effect observed could be due to absorption of preformed hormone from the pituitary graft. To eliminate this possibility, Everett (1956a) conducted a similar experiment on a long-term basis and found a persistence of luteotrophin secretion as long as 120 days post-transplantation.

From this historical review, it can be seen that strong suggestive evidence has accumulated, since the original suggestion by Hohlweg and Junkmann, that the hypothalamus plays an important role in the control of FSH and LH secretion. What effect the hypothalamus has on LTH secretion is still uncertain. Everett (1956a) has suggested that it may be partially inhibitory, since it was observed that LTH is secreted abundantly by anterior lobe grafts which are placed at a distance from the hypothalamus.

In view of the cited observations that luteotrophin secretion is readily demonstrable after transplantation of the pituitary to the kidney, and in view of the fact that most investigators used the anterior chamber of the eye as the site

of transplantation with no report of any luteotrophin secretion, experiments were conducted to find whether pituitary grafts in the eye are capable of secreting this hormone. Also, since Everett confined all of his transplantation operations to the day of estrus, it was of interest to determine whether the time of the cycle at which the pituitary is transplanted has any influence on subsequent luteotrophin secretion.

Inasmuch as the techniques of transplantation of the pituitary gland and of stalk-section with the permanent interruption of the hypophyseal stalk accomplish the same end, i.e., separate the pars distalis from the hypothalamus, it seemed important to investigate whether the effects of the two procedures are similar with respect to FSH, LH and LTH secretion.

Histological studies of pituitary grafts always show that during transplantation considerable loss of parenchyma occurs and also that the differentiated cells disappear. Perhaps these particular elements are more delicate and are quickly destroyed as a result of the operative trauma, thus leaving the graft devoid of highly specialized cells (basophiles) that are presumed normally to secrete FSH and LH. The question arose whether the operative trauma, rather than the separation from the hypothalamus, is sufficient to account for the observed loss of these hormones. To answer this question, grafts were removed from the kidney and re-transplanted under the median eminence of the tuber cinereum, thus producing an additional operative trauma. If following the second transplantation the

graft were to become differentiated again and were to start secreting normally, strong proof would then be provided that the hypothalamus does, in fact, have a definite trophic effect on the pars distalis.

MATERIALS AND METHODS

The experimental animals were adult female rats from the inbred Vanderbilt strain, ranging in age from 4 to 7 months at the beginning of the experiments. The animals, at this time, weighed between 180 and 250 grams. They were kept in an air-conditioned room at a temperature of about 80°F. The lights were controlled by a time switch so that they were "on" for 14 hours and "off" for the next 10 hours. Midnight, "colony time", is considered to be 5 hours after the lights are turned "off"; when the time of the operation is recorded, it is always given in "colony time".

Before the animals were used experimentally, they were kept on a diet of Purina dog chow and water ad libitum. After the various operations were performed, the animals received a supplement of milk three times a week and yeast once a week. Five per cent dextrose was also added to the drinking water.

Vaginal smears were taken at least 2 weeks preoperatively and throughout the postoperative period every day except Sunday. Vaginal smears of the animals used in the re-transplantation series were done every day including Sunday.

1. Operative Procedures.

The hypophysectomy and subsequent transplantation of the pituitary were performed in a single operation. The animals

were hypophysectomized by the parapharyngeal route. The prevention of excess fluid formation in the trachea was accomplished by subcutaneous injection of atropine sulfate (10 mg./kg. body weight) a few minutes before the operation. A polyethylene tube was introduced into the trachea to assure a clear air passage. After a drill hole was placed in the basisphenoid, the whole pituitary gland was removed by suction and transferred into sterile physiological saline. The posterior lobe was separated and discarded. In experiments where the gland was to be transplanted to the kidney capsule, the kidney was exposed at the beginning of the operation and the incision was then temporarily closed. After hypophysectomy the kidney was re-exposed, a small slit was made in the capsule, and the pars distalis was pushed gently through the opening in such a way that the gland was in contact with the kidney surface. The body wall was then repaired.

When the pituitary was to be transplanted to the anterior chamber of the eye, once hypophysectomy had been accomplished a small slit was made with a fine knife near the sclero-corneal junction and the pituitary was introduced through the slit. Because of the restricted operating area, the gland was manipulated considerably more during transplantation to the eye than during transplantation under the kidney capsule.

The sectioning of the hypophyseal stalk and the placing of the pituitary graft under the median eminence were performed using the trans-temporal approach, as described by Harris (1950). After ether anesthesia, a large skin incision ("L-shaped") is

made; one of the limbs of the "L" is extended between the right eye and ear, while the other limb is placed between the two ears. By blunt dissection, the skin is loosened from underlying tissues and retracted, thus permitting the exposure of a large operative field. The temporalis fascia is then cut and retracted. With a blunt instrument, the temporalis muscle is scraped from its bony origin and cut at its insertion into the coronoid process. If tension is placed on this muscle for a few seconds before excision, little or no bleeding occurs at the severed vessels. With the aid of a # 3 dental burr, a large window is made in the skull by removal of a piece of bone extending from the sagittal suture to and below the zygomatic process. The dura mater is then cut and removed, after which the exposed brain can be lifted with a small elevator (Figure 1). When the field is dried, the median eminence and the hypophyseal stalk can be seen in the midline. After the desired experimental procedures, the brain is released, its surface is flooded with sterile saline and blotted, and the skin is sutured to cover the operated area. In the stalk-section experiments, the sectioning was accomplished by breaking the stalk with a very fine hook, and to prevent the regeneration of the portal veins, a barrier was placed between the two cut ends of the stalk*. In re-transplantation experiments, the animals were subjected to surgery

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*The barriers were made from 2 x 4 mm. pieces of oinion skin paper previously dipped in a paraffin bees-wax mixture.

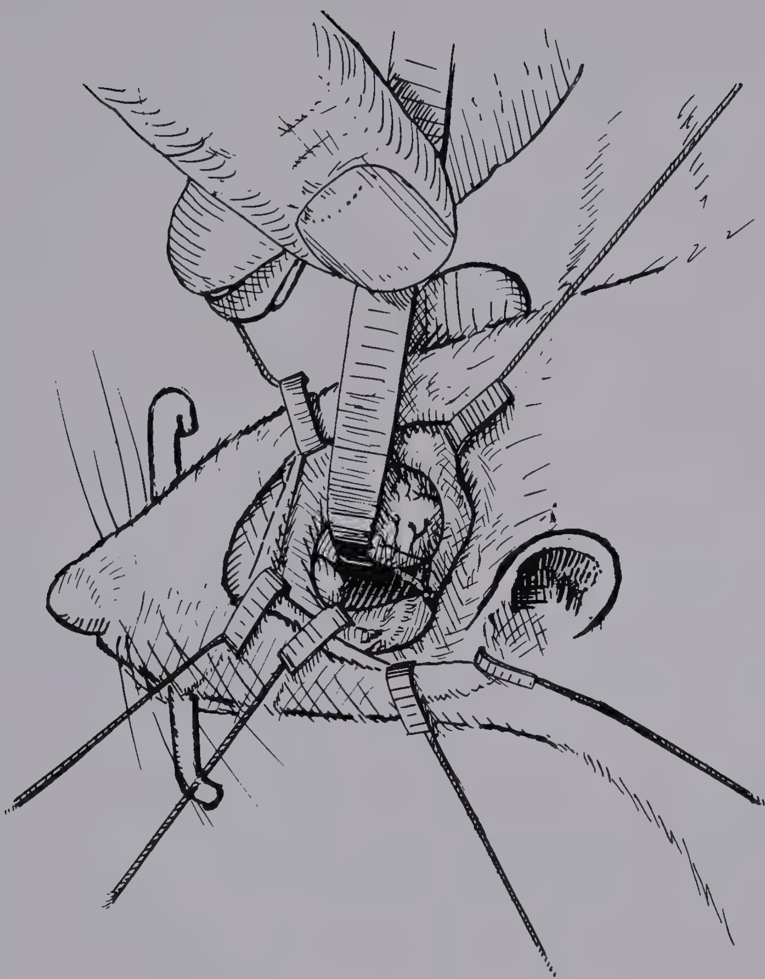

Figure 1. The operative field in the trans-temporal approach to the hypophyseal stalk and the median eminence of the tuber cinereum. The brain is retracted upward to expose the cranial floor.

on two different occasions. During the first operation, they were hypophysectomized and the adenohypophyses autografted to the kidney capsule. Fifteen to 25 days later, the graft was removed from the kidney and placed under the median eminence of the tuber cinereum or under the temporal lobe using the trans-temporal approach described above.

Luteotrophin secretion was tested indirectly by its effect on progesterone secretion by the corpus luteum, as made evident by the deciduoma reaction to uterine trauma. On a given day, two loops of silk thread were tied through the antimesometrial border of one of the two uteri. Four days after the placement of the threads, the degree of decidual reaction was evaluated by the amount of swelling that had occurred around the loops. A minimal reaction was designated by +, a moderate reaction by ++, a good reaction by +++, and a maximal reaction by ++++. In cases of minimal or moderate reaction, serial sections of the region around the loops were prepared, and only if histological proof existed, were these reactions recorded as positive.

2. Autopsy.

At the end of each experiment each animal was killed with an overdose of nembutal. The reproductive tract and the pituitary graft were examined <u>in situ</u> and removed; the ovaries were removed and weighed. In the transplantation series (both to eye and to kidney) the calvarium and cerebral hemispheres were

removed and the base of the skull trimmed to preserve the region of the pituitary capsule.

In the stalk-section groups and in the series of the re-transplantation of pituitary grafts under the median eminence or temporal lobe, the following additional steps were performed after the removal of the ovaries. The animals were perfused with india ink in order to make the hypophyseal portal vessels easily observable. A vasodilating drug, hexamethonium chloride (10 mg.) was injected intravenously into the femoral vein. Immediately, thereafter, the thorax was opened, the ascending aorta cannulated through the left ventricle, and the right auricle cut open. The blood was washed out with 60-80 ml. of physiological saline, followed by 80 ml. of india ink (1:3 dilution in saline). After this injection, the head was removed and the calvarium resected. In the stalk-section experiments, the whole head was fixed. In the re-transplantation series, after the calvarium was resected, the brain was gently lifted and the graft was localized and removed with a block of overlying brain. The base of the skull of these animals was also roughly trimmed at this time.

3. Histology.

All ovaries and other tissues, unless otherwise stated, were fixed in Bouin's solution, imbedded in paraffin and serially sectioned at 10 μ. In the stalk-sectioning experiments, after the head had been fixed in Heidenhain's Susa fixative and decalcified

in 5 per cent trichloroacetic acid (TCA), a block of tissue containing the hypothalamus, the stalk and hypophyseal capsule was trimmed, imbedded in celloidin and cut at thicknesses of 100-200 μ. These sections were mounted without being stained.

In the re-transplantation experiments, the block of tissue containing the overlying brain and graft was imbedded in celloidin, after it had been fixed in Susa. Sections that were alternately thin (6 μ) and very thick (100-160 μ) were then cut by the "dry celloidin" technique. This was repeated until the block of tissue was completely sectioned. The thick sections were mounted without staining; the thin sections were stained differentially.

In those cases where histological study of the pituitary capsule was carried out, the base of the skull containing this area was fixed and decalcified, either by leaving it in Bouin's solution for 5-6 weeks or by fixing it in Susa and decalcifying in 5 per cent TCA. Subsequently this block of tissue was imbedded in paraffin and serially sectioned. All tissues, except the thick celloidin sections, were stained with a modified Mallory trichrome stain.

RESULTS

Part I

1. Pituitary autografts to the anterior chamber of the eye.

This experiment was performed to determine whether the pars distalis when transplanted to the eye behaves the same as it does when transplanted to the kidney capsule. It was found that in 9 out of 14 animals used, the graft was functional and could be seen on direct examination; in the remaining 5, the graft did not "take" and it could not be visualized directly.

A. Animals with functional grafts. The function of the graft and of the corpora lutea was tested by the usual technique of uterine trauma. The test was performed on two occasions on all animals: 5-13 days and 31-70 days, respectively, after transplantation. A positive response to the first trauma would confirm the presence of LTH secretion, and the response to the second trauma would show that the transplant is capable of a prolonged secretion of that hormone. As can be seen in Table I, the first trauma was followed, in all animals, by a maximal decidual reaction. After the second traumatization, however, the results were not as uniform. Two animals responded maximally, three moderately, while four did not respond at all. The appearance of ovaries correlated well with the responses obtained at the two different times of

TABLE I. PITUITARY AUTOGRAFTS TO THE ANTERIOR CHAMBER OF THE EYE

A. Animals with functional grafts

Animal No.	Days from oper. to 1st trauma	Decidual response	Days from oper. to 2nd trauma	Decidual response	Wt. of ovaries (mg.)	Amount of fragment in "Sella"
1	12	++++	40	++++	31.4	----
2	10	++++	31	++++	26.8	very small
3	5	++++	42	++ to +++	23.3	none
4	6	++++	36	++	17.4	none
5	10	++++	31	++	32.8	none
6	13	++++	41	0	29.0	none
7	5	++++	42	0	17.2	none
8	10	++++	36	0	13.5	very small
9	9	++++	70	0	----	large

B. Animals with non-functional grafts

Animal No.	Days from oper. to 1st trauma	Decidual response
1	5	0
2	12	0
3	12	0
4	13	0
5	10	0

traumatization. Early in the experiment, at the time of the first traumatization, the ovaries of all animals contained full sets of large corpora lutea (approx. diameter, 1.5 mm.). At autopsy, which was performed 4 days after the second trauma, the ovaries of animals that responded with a decidual reaction always contained several larger corpora lutea (Figure 2), while the remaining animals did not. Their ovaries were atrophic and bore only small corpora lutea. The average weight of the ovaries was 21.4 mg. (range: 13.5 to 32.8 mg.). The histological examination confirmed the observations made at autopsy. It also showed clearly that the follicular apparatus and the intersitial tissue were atrophied. The interstitial cells were small and the "wheel nuclei", characteristic in this species of the lack of LH secretion, could be seen. The pituitary grafts showed the following histological features: in animals in which the corpora lutea were non-functional, the grafts were always small and very poorly vascularized; grafts taken from animals that had functional corpora lutea were always larger and better vascularized. By comparison with grafts in the kidney, however, they were distinctly smaller and had less prominent sinusoids. Serial sections of pituitary capsules showed either the presence of no fragments of adenohypophysis or of very small remnants (when present, such remnants comprised clusters of only few cells, which appeared undifferentiated). In one case only (animal # 9) there was a somewhat larger fragment in which a few cells were differentiated. This animal has been included in the series because it is believed that even

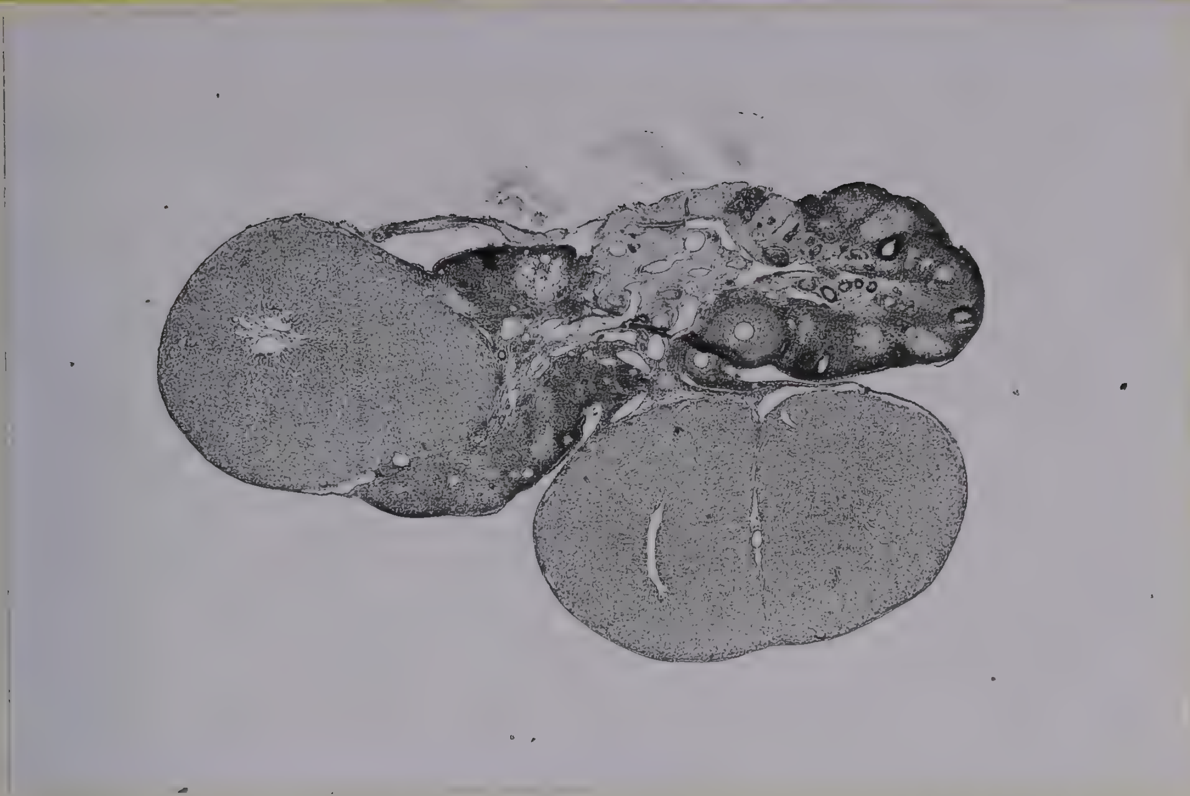

Figure 2. Ovary from a rat with a pituitary graft in the eye. Note the large corpora lutea and atrophied interstitial tissue and follicular apparatus. x 30.

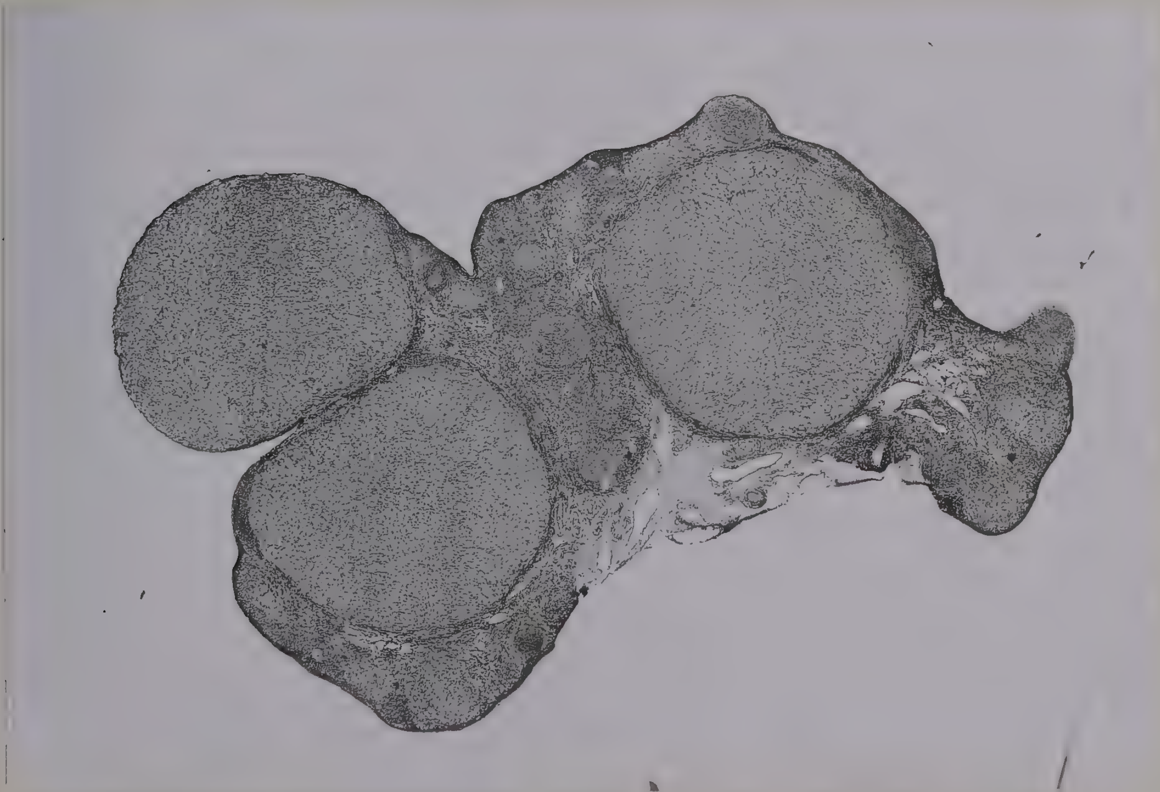

Figure 3. Ovary from a rat with a pituitary graft in the kidney. Transplantation on diestrus day 2. Same features as Figure 2. x 30.

this fragment was too small to have any influence on the ovary. The ovarian histology of this animal did not differ from the others in the group. Also, daily vaginal smears of this animal, like the others in the group, showed a continual diestrous state throughout the experimental period.

B. Animals with non-functional grafts. In each of the 5 animals in this group, one traumatization was performed 5-13 days after transplantation. As can be seen from Table I, none of the uteri responded with a decidual reaction. The animals were sacrificed 17-20 days after transplantation and at that time, all the ovaries were very atrophic and contained only small corpora lutea. At autopsy, the eyes bearing the grafts were examined with a dissecting microscope and, in no case, could any appreciable amount of glandular tissue be found. The base of each skull was carefully searched in this manner, and no remnants of pars distalis could be seen.

2. Pituitary autografts to the kidney capsule.

This experiment was performed to find out whether the anterior pituitary graft transplanted to the kidney on different days of the cycle will behave the same, i.e., secrete LTH, as it does when transplanted on the day of estrus.

A. Transplantation on the day of estrus or on the second day of diestrus. Two of the 9 animals in this group were operated on the day of estrus (Stage III) and the other 7 on the second day of diestrus (Stage V_2). In the majority of animals, the function

of the graft was tested by uterine trauma on two occasions: 3-14 days and 17-37 days, respectively, after the pituitary was transplanted. As can be seen from Table II, the decidual response to the second trauma was very similar to that of the first trauma. Throughout the experiment, the ovaries retained the same appearance, always having several large and healthy corpora lutea. The rest of the ovary was consistently small with atrophic interstitial tissue and no antral follicles. Except for one animal (diestrus transplantation), ovaries in this group were examined exclusively with the dissecting microscope (7x). The one case in which the histological study was carried out is represented in Figure 3. One can see the persisting corpora lutea, the scanty interstitial tissue and the complete atrophy of the follicular apparatus. The general aspect of ovaries in the group transplanted during diestrus is so very similar to the ovaries described by Everett (1954, 1956) after transplantation during estrus, that no additional operations during estrus seemed necessary at this time.

B. Transplantation in proestrus or late diestrus. This group can be subdivided into two subgroups, in both of which the actual time in days between the last ovulation and transplantation was identical, but which differed as follows: (B_1) this consists of animals with a history of 4-day cycles. Their anterior pituitaries were transplanted on the day of proestrus. (B_2) animals with cycles of 5-day duration were used and here the pituitary was transplanted on the third day of diestrus.

Differences and similarities encountered in the two groups

TABLE II. PITUITARY AUTOGRAFTS TO THE KIDNEY CAPSULE

Transplantation done on the day of estrus or second day of diestrus

Animal No.	Day of cycle	Days from oper. to 1st trauma	Decidual response	Days from oper. to 2nd trauma	Decidaul response
1	III	8	++++	22	++++
2	III	13	++++	37	++++
3	V_2	13	++++	37	++
4	V_2	3	++++	17	++++
5	V_2	3	++++	17	++++
6	V_2	11	+++	35	+++
7	V_2	11	++++	35	+++
8	V_2	6	+++	35	--
9	V_2	7	++++	--	--

will now be considered. Group (B_1) In the 15 animals in this group, the removal of the adenohypophysis and its subsequent grafting to the kidney capsule was done between 8:30 A.M. and 1:45 P.M. The time given indicates the beginning of the operation which was therefore carried out before the occurrence of LH release (Everett, 1956b). Four to 21 days following this operation, the right uteri of all animals were traumatized. The uteri in 12 of the 15 animals did not respond with a decidual reaction.

To test the possibility that the grafts were nevertheless secreting luteotrophin, new corpora lutea were artificially induced by injections of FSH and LH*. The rationale of this procedure is as follows: if these newly formed corpora lutea became sufficiently stimulated to produce a deciduoma reaction to uterine trauma, this would conclusively show that the pituitary graft was secreting LTH and that the negative result of the first trauma was due to a lack of responsive corpora lutea and not to a lack of LTH secretion.

Some preparations of FSH and LH were not effective in the doses administered and did not produce new corpora lutea. For this reason, 4 animals (## 2, 4, 8 and 9) were given an additional series of injections of FSH and LH. However, this was successful in only 2 animals (## 8 and 9). It can be seen in Table III that, except for the first 4 animals, the second trauma was followed

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*The FSH injections were begun 23-67 days after pituitary transplantation. Depending on the hormone preparation used, 1 to 5 mg. was injected subcutaneously twice daily during a period of 3 days. This was followed on the fourth day by an injection of LH.

TABLE III. PITUITARY GRAFTS TRANSPLANTED TO THE KIDNEY CAPSULE ON THE DAY OF PROESTRUS

Animal No.	Time of oper.	Days from oper. to 1st trauma	Decidual response	Days from oper. to injection FSH	LH	Days from oper. to 2nd trauma	Decidual response	Wt. of ovaries (mg.)
1	-before--11:00---A.M.----	4	0	---	---	---	---	---
2*		4	0	67 to	70	---	---	12.4
3		7	0	33 to	36	41	0	25.7
4*		6	0	32 to	35	54	0	29.8
5		6	0	32 to	35	40	+ to ++	33.8
6		12	0	46 to	49	58	++++	37.7
7		21	0	29 to	32	37	++++	33.1
8*	12:30	21	0	29 to	32	48	++ & +++	31.0
9*	12:55	19	0	27 to	30	46	++	21.5
10	12:15	15	0	23 to	26	37	+++	20.8
11**	1:45	5	0	31 to	34	40	++ & +++	16.9 (one)
12**	12:30	13	0	20 to	23	29	++++	38.1 (one)
13	before 11:00	7	++ to +++	---	---	---	---	---
14	12:55	15	++ to +++	---	---	---	---	24.2
15	1:35	15	++ to +++	---	---	---	---	24.5

*An additional series of hormone injections was given, not listed in Table (see Text).
**Right ovary was removed 4 days after right uterus was traumatized.

with a moderate to maximal response, including ## 8 and 9 which received the additional series of hormonal injections.

Group (B_2) In the 9 rats in which pituitary transplantation was carried out on the third day of diestrus in 5-day cycles, the right uterus was traumatized 4 to 10 days after transplantation and gave a maximal or nearly maximal response uniformly (Table IV). This shows that there is a significant difference between animals in this group and in the preceding one. Although the ages of corpora lutea in the two groups are the same, a distinct difference must exist between the hormonal state on the day of proestrus and on the third day of diestrus in order to account for the observed results.

The gross appearance of ovaries in Groups (B_1) and (B_2) at the time of the first trauma did not greatly differ. Both had inconspicuous interstitial tissue and arrested follicular development. Both had full sets of corpora lutea, but in Group (B_1) these were uniformly small (1.2 mm. approx. diameter), while in Group (B_2), occasional corpora lutea of somewhat larger size were found. Although the corpora lutea in the two groups did not otherwise differ in external appearance, functionally they clearly differed as demonstrated by deciduomata in Group (B_2) and their absence in Group (B_1). In two animals from Group (B_1) the right ovaries were removed at the time of the first trauma (preceding FSH and LH treatment) and, by histological technique, were compared to the ovaries of Group (B_2) (Figures 4 and 5). Only when careful microscopic study of the lutein tissue was carried out was it apparent that a greater difference, than was appreciated on gross examination,

TABLE IV. PITUITARY AUTOGRAFTS TO THE KIDNEY CAPSULE

Transplantation on the third day of diestrus

Animal No.	Days from oper. to 1st trauma	Decidual response	Days from oper. to autopsy	Wt. of ovaries (mg.)
1	6	+++	10	29.1
2	5	+++	10	42.2
3	4	++++	9	33.4
4	10	++++ & +++	17	---
5	6	+++	19	---
6	9	++ & +++	13	42.8
7	9	++++	13	31.7
8	5	++++	44	19.3
9	6	++ & +++	32	21.5

existed between the corpora lutea of the two groups. The lutein cells in Group (B_2) were large, healthy and well organized (Figure 7) and looked very similar to the cells of corpora lutea in the group of animals operated on the day of estrus or second day of diestrus (Figure 6). On the contrary, the lutein cells of Group (B_1) were small, often fatty and the whole corpus luteum was invaded by numerous stroma cells (Figure 8). This structural aspect is in agreement with the non-functional state of these corpora lutea.

At autopsy, the appearance of the other ovaries in Group (B_1) varied with the degree to which these glands were stimulated by the injected hormones. In those animals that had not responded to the injected FSH and LH the ovaries were atrophic, while in others a varying number of corpora lutea were present. The corpora lutea were usually large and well vascularized. The weight of these ovaries differed with the number of corpora lutea present, as can be seen from Table III. In the majority of cases the weight was in line with those of Group (B_2), whereas there is little doubt that they would have been completely atrophic were it not for the FSH and LH treatment. Some of the ovaries were serially sectioned and it was found that the new corpora lutea produced by FSH and LH treatment now looked very healthy, but that the interstitial tissue and follicular apparatus remained largely atrophic.

In the days immediately following the operation, there were some small variations in the smear picture among the animals

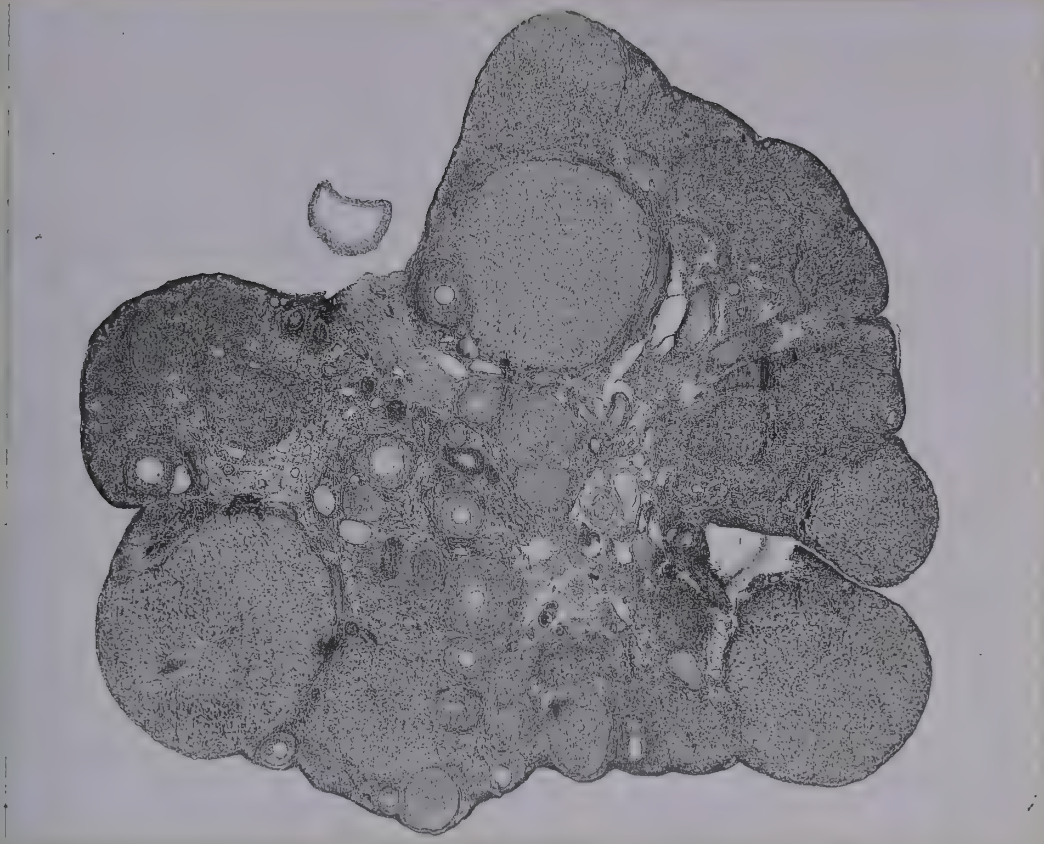

Figure 4. Ovary from a rat with a pituitary graft in the kidney. Transplantation on diestrus day 3. Corpora lutea smaller than in Figures 2 and 3. x 30.

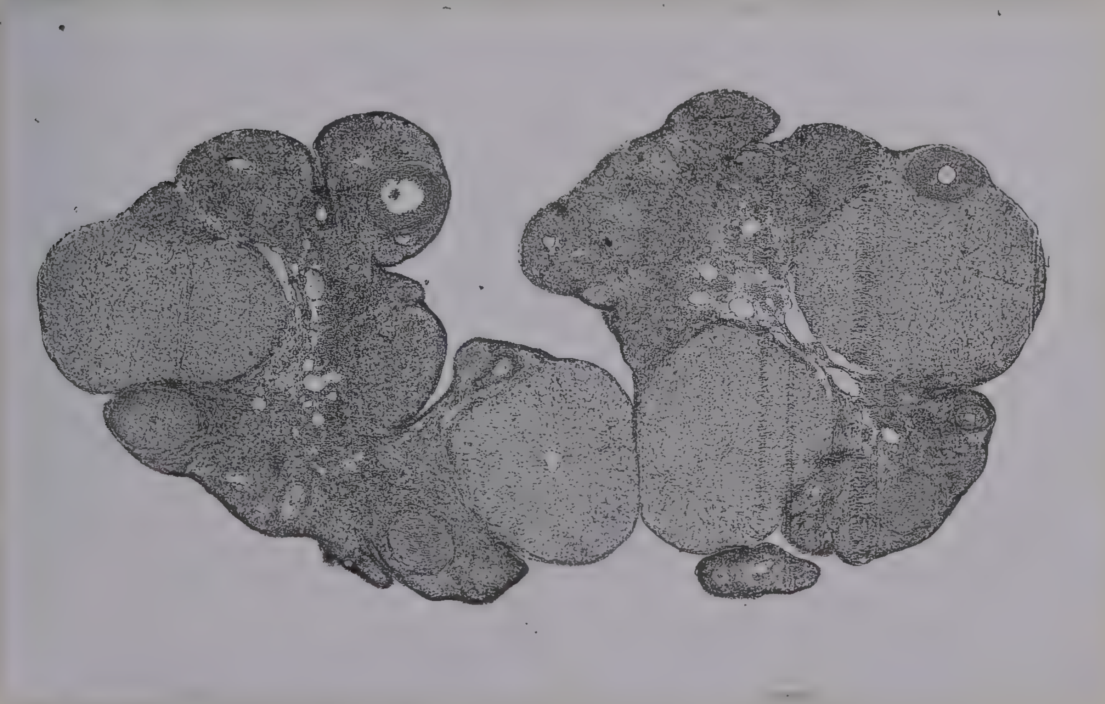

Figure 5. Ovary from a rat with a pituitary graft in the kidney. Transplantation in proestrus. Note the great similarity with Figure 4. x 30.

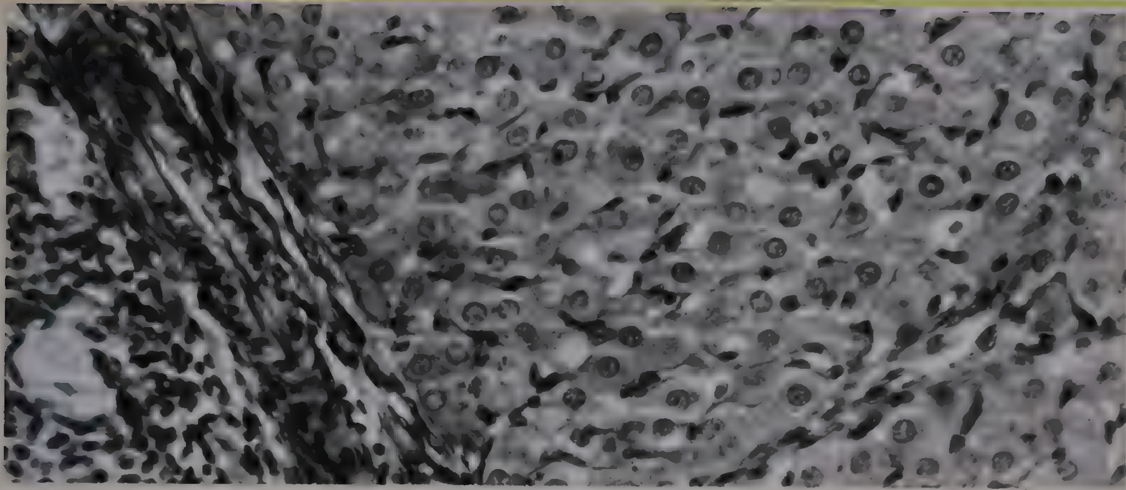

Figure 6. Luteal cells from ovary shown in Figure 3. Pituitary transplantation on diestrus day 2. x 400.

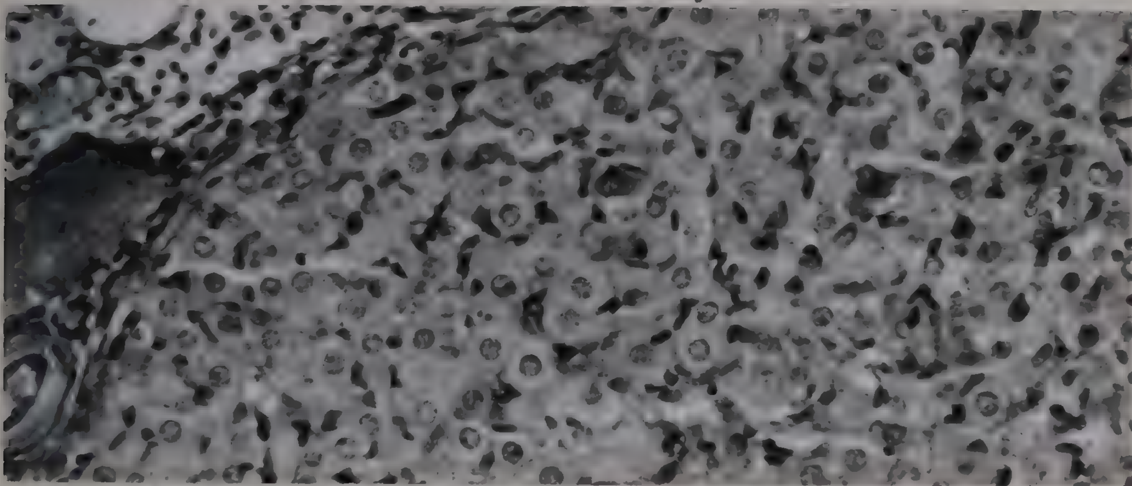

Figure 7. Luteal cells from ovary shown in Figure 4. Pituitary transplantation on diestrus day 3. Note the great similarity with Figure 6. x 400.

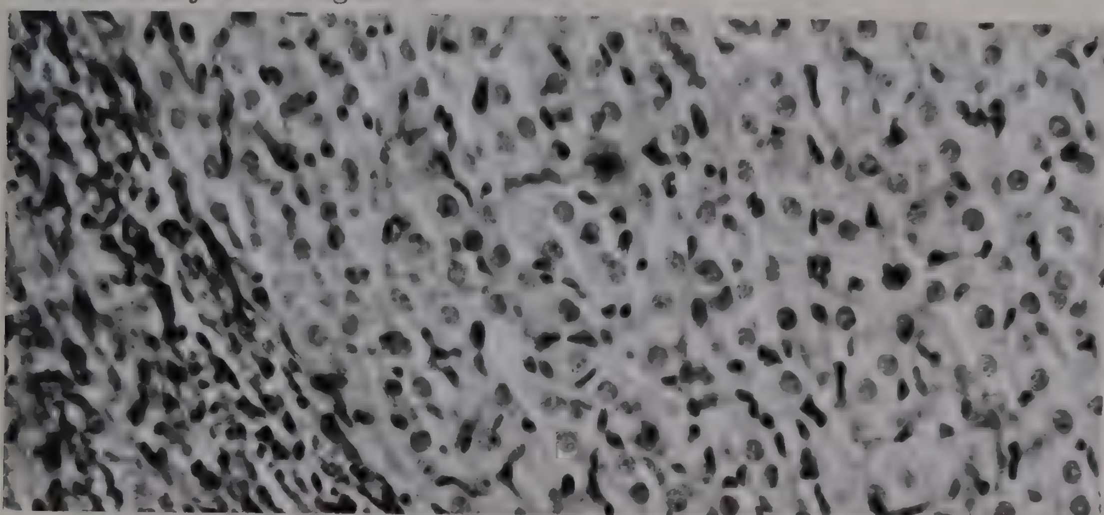

Figure 8. Luteal cells from corpora lutea in Figure 5. Pituitary transplantation in proestrus. Contrast with Figures 6 and 7 and note smaller luteal cells and increased stroma. x 400.

of different groups, depending on the stage of the cycle at which the pituitary transplantation was carried out. Otherwise, however, the smears remained of the diestrous type, except for a transient estrogenic response to the FSH-LH injections in Group (B_1).

Part II

Transection of the Pituitary Stalk

This part of the experiment was performed in order to find out whether the stalk-sectioning in itself could produce pseudopregnancy and whether this pseudopregnancy could be prolonged indefinitely by keeping the anterior pituitary well separated from the hypothalamus.

A total of 22 animals were used for this experiment and were divided into four groups as follows: Group A consists of 7 animals in which the stalk was sectioned and a barrier placed so that it effectively prevented any regeneration of the hypophyseal portal vessels. Group B consists of 9 animals in which a barrier was defectively placed so that regeneration of the portal veins was possible. Group C is represented by 3 animals in which the brain was lifted very near the median eminence (as when the stalk-section is actually performed) but the stalk was neither cut nor manipulated. Group D is also represented by 3 animals which were treated similarly to Group C but in which the cerebral hemisphere was lifted in such a way as to completely avoid the region of the hypothalamus. All operations were performed on the day of estrus except for one animal in each of Groups A and B, in which the operation was performed during

diestrus. The results are as follows:

Group A. In all 7 animals one uterus was traumatized 4 to 35 days after the operation. In 6 animals the other uterus was treated similarly 46 to 74 days after sectioning the stalk. The decidual responses to the first uterine traumatizations were maximal in all animals except two (## 5 and 6). The responses to the second traumatizations were moderate to maximal except in the same above mentioned two animals (Table V). The histological examination of the decidual reaction following the second traumatization showed that this was in no way different from the deciduomata obtained in normal rats made pseudopregnant by the stimulation of the cervix (Figure 9). In the two animals (## 5 and 6), whose ovaries possessed large corpora lutea but whose uteri were not reactive to traumatization, the vaginal response to an excess of estrogen was determined*: Progesterone will mucify the epithelium in spite of the estrogen, which otherwise would cause a prolonged cornification (Allen and Meyer, 1935, Astwood, 1941, Desclin, 1950, Everett, 1956). The responses of these two animals to the estrogen test were such that they produced a definite mucified smear, and only a scanty number of cornified cells could be seen. Thus, although the decidual

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*The procedure used for these two animals was to inject subcutaneously, on a given day, 50 µg. of estradiol-benzoate (EBZ) and to repeat the injection 3 days later. On the third day thereafter the animals were sacrificed.

TABLE V. TRANSSECTION OF THE PITUITARY STALK

Animal No.	Days from oper. to 1st trauma	Decidual response	Days from oper. to 2nd trauma	Decidual response	Days from oper. to Autopsy	Wt. (mg.) ovaries	Wt. (mg.) adrenals	Days of anestrus after oper.
Group A								
1	29	+++	74	++++	78	35.8	12.0	---*
2	5	++++	49	++++	54	27.3	14.8	---*
3	7	+++	46	++ to +++	51	33.1	17.0	---*
4	34	+++	52	++	56	30.4	16.2	---*
5	35	0	53	0	61	54.0	15.1	---*
6	35	0	53	0	61	57.5	14.7	---*
7	4	++++			61	41.2	14.9	---*
Group B								
1	15	0			41			36
2	10	0			30			15
3	8	0			31	51.3	35.5	16
4	10	0			30			15

		Group B (continued)		
5	4	++++	61	27
6	---	---	27	16
7	---	---	26	20
8	---	---	26	16
9	---	---	23	19
		Group C		
1	5	+++	9	
2	4	++++	35	16
3	4	+++	21	20
		Group D		
1	5	0	9	cycles not interrupted
2	5	0	9	"
3	4	0	35	18

*None of these animals showed any vaginal cyclic changes throughout the experimental period.

Figure 9. Section of uterus showing a good decidual reaction produced by traumatization 74 days following stalk-section with a well-placed barrier. x 20.

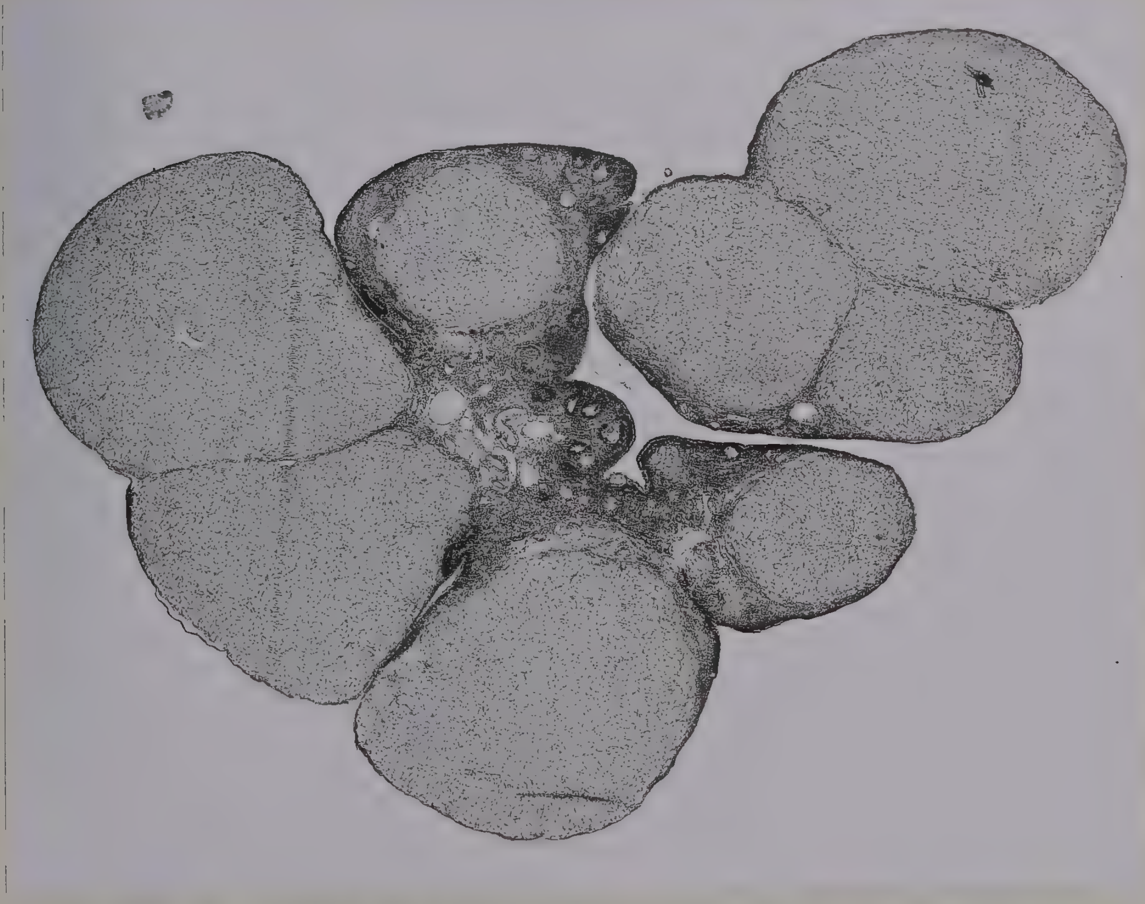

Figure 10. Ovary from a stalk-sectioned rat having an effective barrier to vascular regeneration. Note the greater size of the corpora lutea, compared with those in Figures 2 and 3. x 30.

reactions had failed, the corpora lutea were functional, nevertheless.

All of the animals in this Group had a complete absence of estrous cycles and showed a diestrous smear from the time of operation until autopsy.

The gross appearance of the ovaries changed very little in the course of the experiment, appearing at the time of autopsy much as they had at the time of the first traumatization. All had a number of large corpora lutea, the interstitial tissue was atrophied, and any follicles seen were very small (Figure 10). The histology of these ovaries is much like that described for the animals in which the pituitary was transplanted on the day of estrus or second day of diestrus (as can be seen by comparing Figures 10 and 3) the only difference being that the corpora lutea in the ovaries of the stalk-section series (Figure 10) are somewhat larger. The average ovarian weight, excluding animals #5 and #6, was 35.6 mg. (range: 27.3 to 46.0 mg.).

A study of the serially mounted, thick sections of the region of the stalk disclosed that, in all cases, the barrier was very well placed and although the pars distalis was well injected with india ink, no regeneration of the hypophyseal portal vessels could be seen (Figure 11). The posterior lobe, in all cases, was degenerated and very scantily vascularized.

Group B. Of all 9 animals which comprise this group, in only 5 was the function of the corpora lutea tested. This was

done with a single traumatization 4 to 15 days after the stalk-section. Animal # 5, whose right uterus was traumatized 4 days after the operation, exhibited a maximal decidual reaction. The other 4 animals, traumatized at a later date, gave no response.

In the days immediately following the operation, the vaginal smear showed a picture of complete diestrus. As can be seen in Table V, the diestrous smear was later replaced by cycling variations starting 15 to 36 days after the operation.

The appearance of the ovaries, at the time of trauma, was uniform. They were large, contained large corpora lutea (approx. diam. 1.5-1.8 mm.), the interstitial tissue appeared abundant and healthy, and a great number of prominent follicles could be seen.

In 4 of 9 animals, thick serial sections of the region of the stalk disclosed that, in all cases, regeneration of the portal blood vessels had taken place (that these vessels regenerate well is shown in Figure 12). The barrier was placed too anteriorly and just reached the anterior extremity of the gland; in the sections, vessels can be seen going around the barrier. In the remaining 5 animals, no serial sections were made, but at autopsy the region of the stalk was carefully examined under the dissecting microscope, and here also the barrier was similarly displaced.

Group C. In this group of 3 animals in which the brain was lifted close to the hypothalamus but the stalk was not sectioned, the right uteri were traumatized 4 or 5 days later.

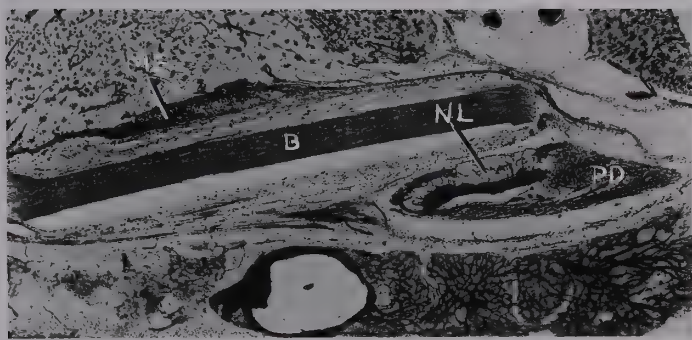

Figure 11. Sagittal section through the median eminence (ME) and hypophysis showing a well-placed barrier (B). Note the well-injected pars distalis (PD) and the very scantly vascularized neural lobe (NL). India ink injection. x 20.

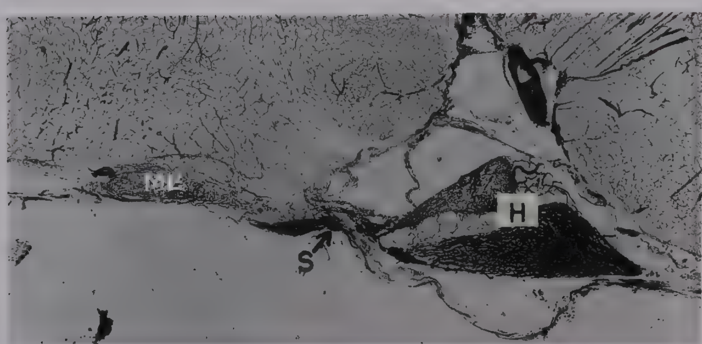

Figure 12. Sagittal section through the median eminence (ME), hypophyseal stalk (S) and the hypophysis (H). At arrow the stalk had been sectioned. Note the re-establishment of vascular connections between gland and hypothalamus. India ink injection. x 20.

The decidual responses were maximal or nearly so. At that time, the ovaries closely resembled those in Group B, possessing large corpora lutea, apparently healthy interstitial tissue and a number of prominent follicles in different stages of development. As in Group B, the vaginal smears on the days immediately following the operation were diestrous. The reappearance of cycling changes was recorded 16 and 20 days after the operation in the two surviving animals.

<u>Group</u> <u>D</u>. This group was treated in the same way as Group C except the hypothalamic region itself was not disturbed. Only more lateral parts of the brain were elevated. In contrast to Group C, uterine trauma here failed to cause a decidual reaction in any case. In fact, two animals continued normal cycles without interruption. The third animal had a long period of diestrus.

<u>In comparing the results of the four groups in this series</u>, it is seen that simple mechanical manipulation of the hypothalamus (Group C) is sufficient to <u>initiate</u> the state of pseudopregnancy. Whether this will <u>persist</u> depends on how complete is the separation of the anterior pituitary from the hypothalamus and median eminence. If the separation remains complete, a continuing pseudopregnancy is established (Group A). When regeneration of the portal vessels is allowed by faulty placement of the barrier (Group B), the pseudopregnancy ends with the re-establishment of hypothalamo-pituitary

connections. Non-specific trauma (Group D) does not in itself produce pseudopregnancy.

Part III

Re-transplantation Experiments

In this phase of the study, the pituitary graft, after being in the kidney for at least a fortnight, was re-transplanted under the median eminence of the tuber cinereum. This was done in order to test the direct influence of the hypothalamus on the graft.

The experimental group consisted of 14 animals; the control group of 12 animals. The respective operative procedures were as follows: The first was the same for both groups and consisted in transplantation of the anterior pituitary to the kidney. In the second procedure (re-transplantation) the graft was removed from the kidney and placed under either the hypothalamus or under the temporal lobe of the brain. Both groups of animals were observed for periods up to five and one-half months. Special attention was paid to vaginal smears, since it was expected that any marked stimulation of gonadotrophin secretion would be detectable by that means.

When no changes had occurred after a few weeks, the animals were primed with small amounts of estrone (5-15 µg. subcutaneously in oil)*.

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*The assumption was made that the hypothalamus may have regressed to a "prepubertal" status. Female rats experienced early puberty and early cycling after receiving such treatment at 25 days of age.

In one animal, after a single injection of 10 µg. of estrone, cycling vaginal changes began within the week. In other animals, this treatment was repeated 2 to 5 times at irregular intervals before cycles reappeared.

Out of a group of 14 animals in which the pituitary graft was found to be well placed under the median eminence (Figures 13 and 14), 5 showed cyclic vaginal changes. These cycles were, for the most part, of 6 to 7 days duration and frequently included periods of cornification lasting for 2 days. In the remaining animals in this group, and in all of the control group, estrone injections produced no estrous cycles.

The 5 cycling animals were placed with males, and 3 of them became pregnant. On the twenty-third day of pregnancy, each litter was delivered by caesarian section. Only one animal attempted spontaneous delivery and this was unsuccessful. The litters were all small in number, consisting of 5 pups in two cases and 2 pups in one case. The young were all viable at the time of delivery and were exceptionally large. The placentas were also noticeably enlarged, compared to normal ones at term.

The other 2 animals remained infertile; in fact, they usually failed to copulate with known fertile males in repeated trial matings. Progesterone therapy (1 mg. in oil) on the afternoon of proestrus was twice followed by infertile mating in each rat, as indicated by the presence of sperm in the next day's vaginal smear. The absence of even pseudopregnancy requires

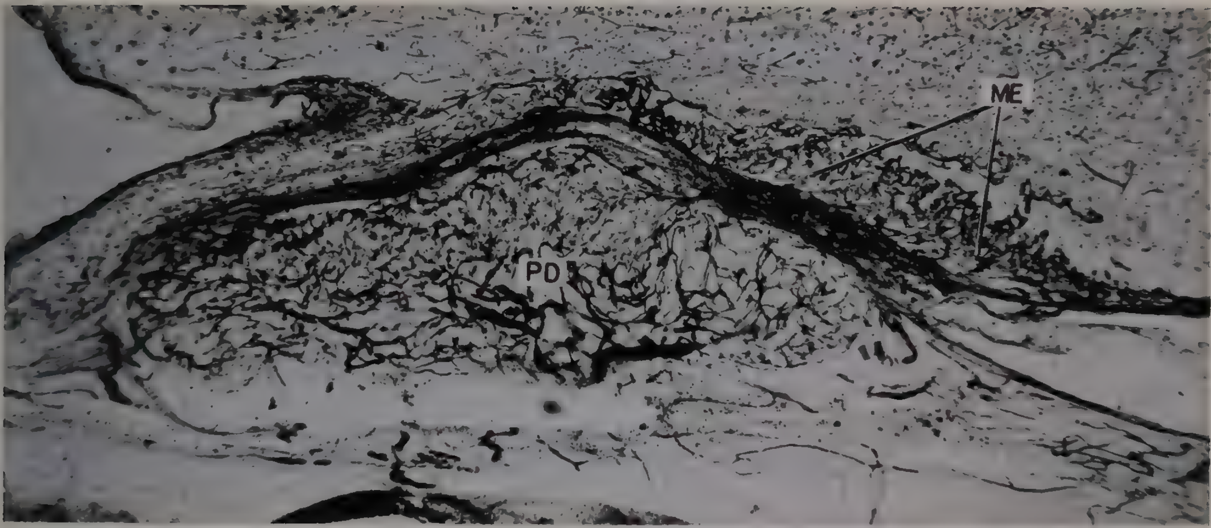

Figure 13. Sagittal section which shows the vascular connections between the median eminence (ME) and the retransplanted pars distalis (PD). 100 u celloidin, unstained section. India ink injection. x 50.

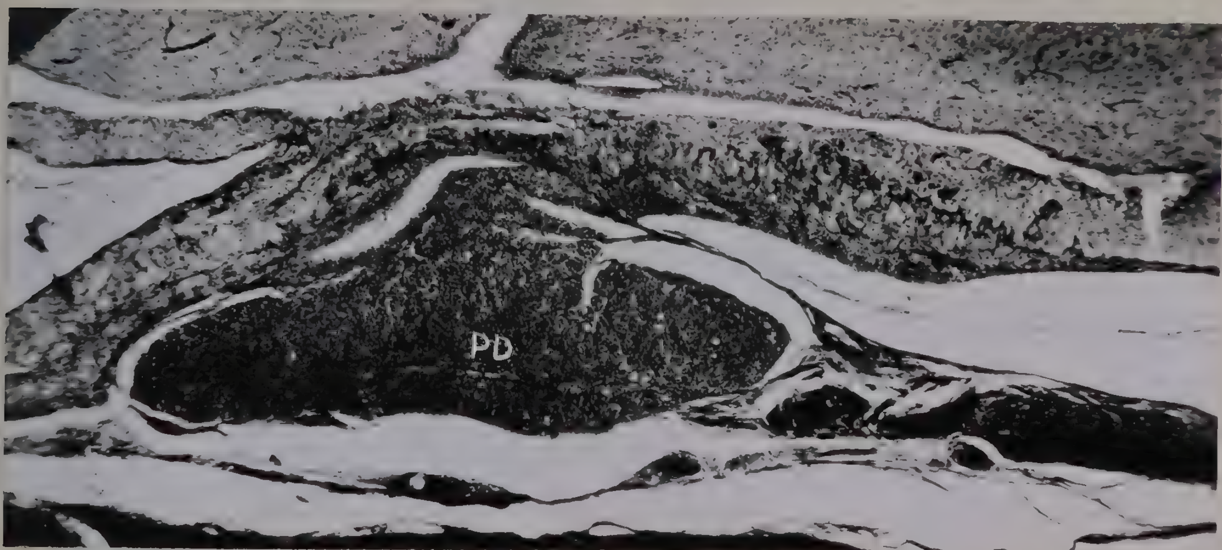

Figure 14. Thin sagittal section near the preceding one. Note the rather large size of the graft (PD). Modified Mallory stain. x 50.

comment; perhaps it is to be correlated with the very small number of corpora lutea produced in these particular animals.

At the end of the various experiments the following observations were made:

Ovaries. The complete disappearance of the large, persisting corpora lutea, which were known to be present at the time of re-transplantation, was a common feature of all ovaries. From the weights and appearance of ovaries, all animals could be divided into two groups, the first group comprising the 5 cycling animals, the second group, the remaining ones. In the first group, the ovaries had an appearance characteristic of the time of the cycle in which the animal was killed. In general, the fdlicular apparatus was moderately developed, fresh corpora lutea were present, and some repair of the interstitial tissue was seen (Figure 15). In the second (non-cycling) group of animals, the ovaries showed, uniformly, a picture of complete atrophy of both the follicular and interstitial tissue and a lack of corpora lutea (Figure 16). In the cycling animals, the average ovarian weight was 25.5 mg. (range: 20.6 to 30.6 mg.). This weight, although not equal to that of normal ovaries is, nevertheless, far above that of the ovaries of the non-cycling animals, which was 7.4 mg. (range: 4.2 to 11.7 mg.).

Uterus and Vagina. Histological study showed complete atrophy of the mucosa in non-cycling animals and this atrophy

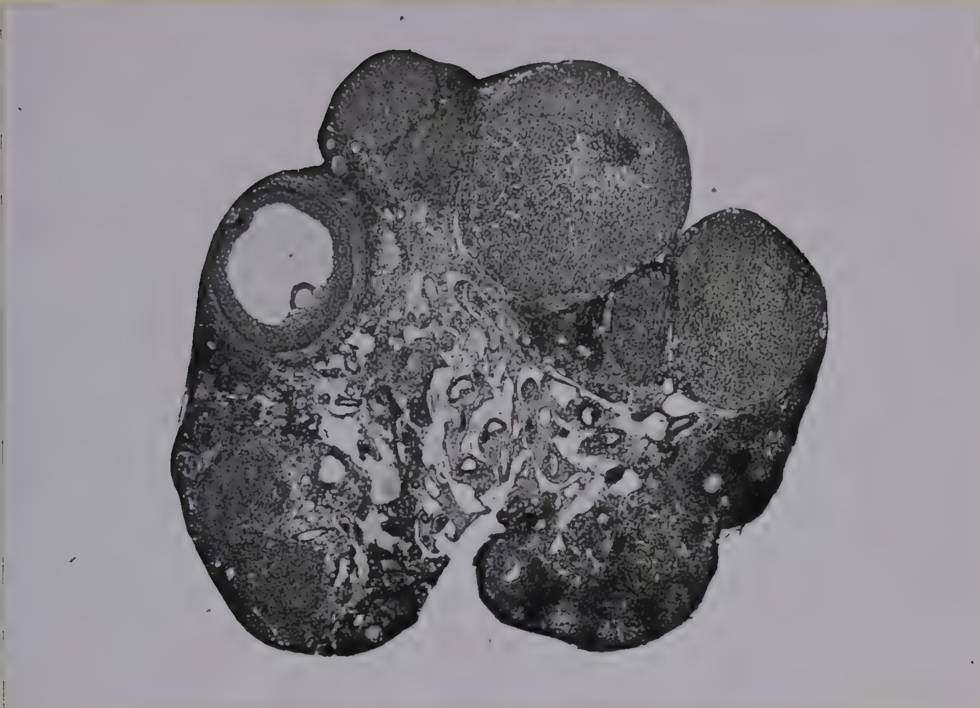

Figure 15. Ovary from a rat that had re-cycled following re-transplantation of the pituitary graft under the median eminence. Animal was killed during proestrus. Note the presence of cyclic corpora lutea and a large graafian follicle. x 30.

Figure 16. Ovary from a rat following re-transplantation of the pituitary graft under the temporal lobe. Complete atrophy of the organ is evident. x 30.

was well repaired in the animals of the re-cycling group. In Figures 17 and 18, sections of uteri in the non-cycling and cycling groups, respectively, are represented. When these are compared, the striking difference is evident. In Figure 17 the uterine epithelium is low cuboidal like that found in castrated animals, while in Figure 18 (re-cycling group) it is seen that this epithelium is columnar and presents the typical picture of proestrus. The same is true for the appearance of sections of vaginae. The mucosa is very thin and shows an epithelium which is severely atrophic in the non-cycling group (Figure 19). On the other hand, the vaginae in animals from the cycling group show marked repair (Figure 20); the stratified squamous epithelium lining the lumen of the organ is indistinguishable from that found in normal animals.

Pituitary grafts. Histological sections of the graft, whether it was placed under the median eminence or under the temporal lobe, showed that an additional loss of parenchyma had occurred since re-transplantation. However, the impression was that the loss was greater in the grafts that had been placed under the temporal lobe. That the graft can still be of considerable size is seen in Figures 13 and 14 which represent a gland well-placed under the median eminence. Vascularization as studied in thick celloidin sections, appeared equally well developed in either location. Cytologically, however, there was a great difference. In all temporal lobe grafts the

[illegible] in the [illegible] of the re-cycling group. In Figures 17 and 18, [illegible] of uteri in the non-cycling and [illegible], [illegible], are represented. [illegible] [illegible], the atrophic difference is evident. In Figure 17 the uterine epithelium is low cuboidal like that [illegible] [illegible]) [illegible] that the epithelium is columnar and [illegible] [illegible] [illegible] of vacuoles of varying [illegible]. The mucosa is very thin [illegible] epithelium which is severely atrophic in the non-cycling [illegible] (Figure 18). On the other hand, the vaginas [illegible] from the cycling group show marked repair (Figure [illegible]); the stratified squamous epithelium lining the lumen of the [illegible] indistinguishable from that found in normal animals.

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[illegible] claimed that an additional loss of [illegible] and since pre-treatment. However, the important [illegible] the temporal lobe. That the [illegible] can still be of con-[illegible] [illegible] placed under the [illegible] [illegible]. The [illegible] or [illegible] thick cellular sections, appeared equally well [illegible] [illegible]. In all [illegible] I [illegible] the

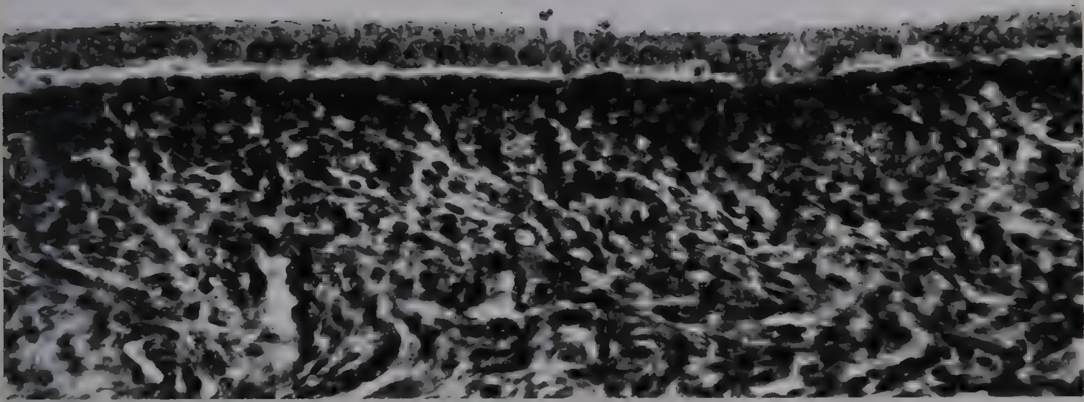

Figure 17. Uterine mucosa from a rat following re-transplantation of the pituitary graft under the temporal lobe. Complete atrophy. x 400.

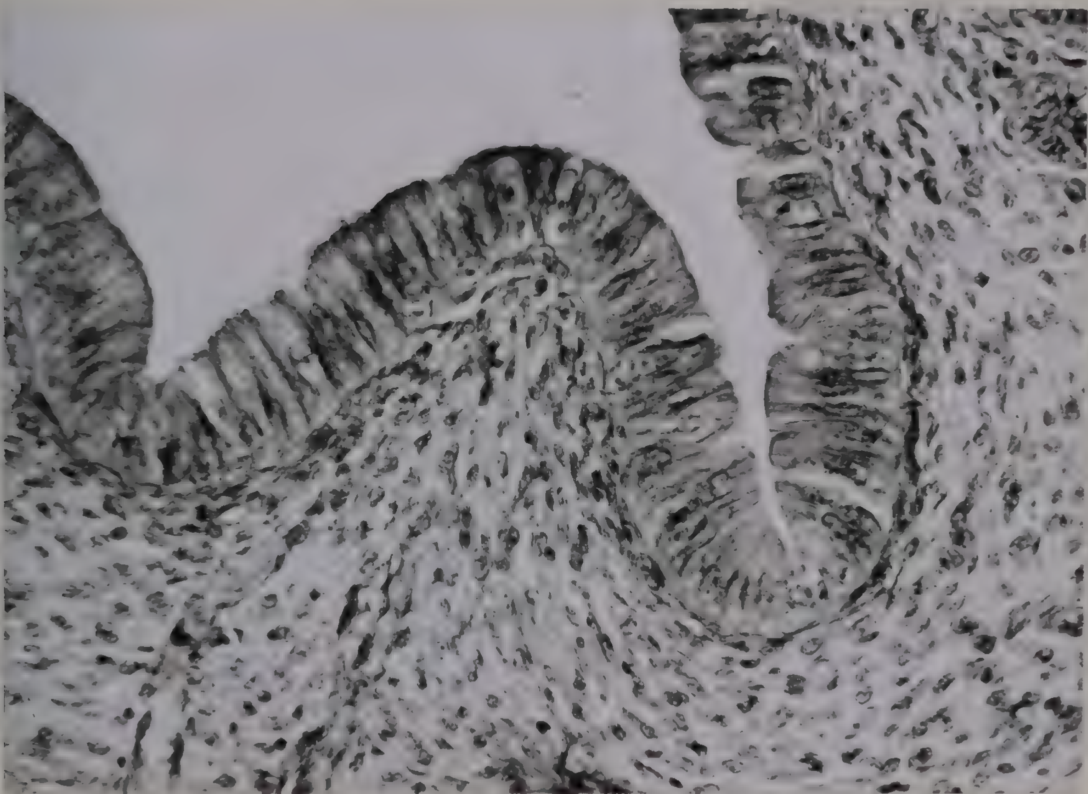

Figure 18. Uterine mucosa from a rat that had re-cycled following re-transplantation of the pituitary graft under the median eminence. Note maximal repair. x 400.

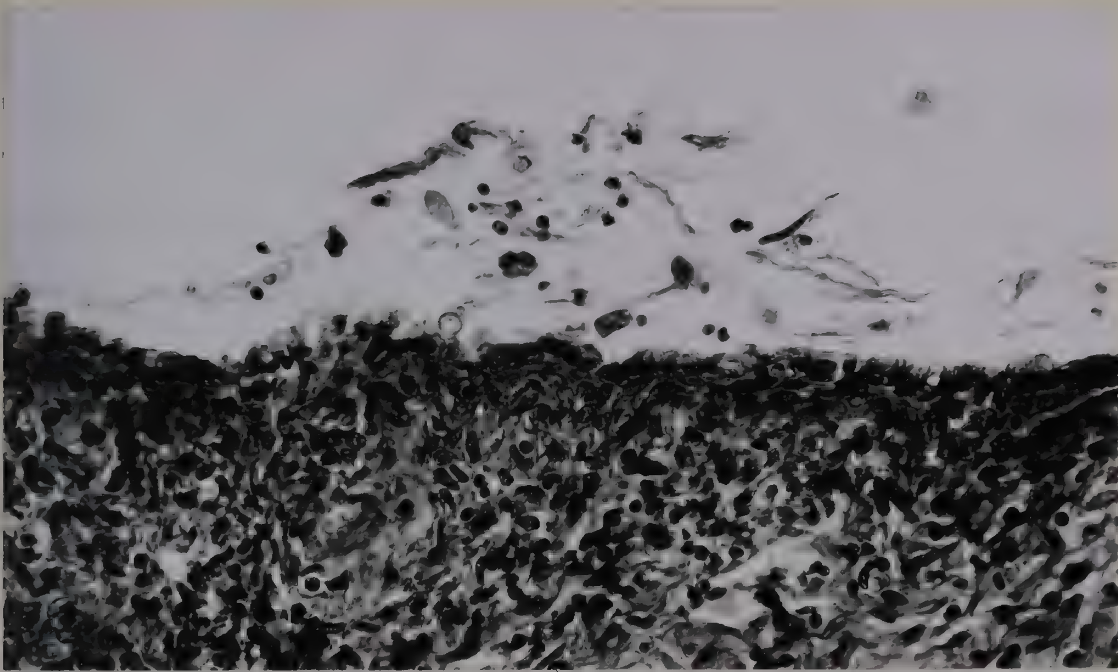

Figure 19. Vaginal mucosa from a rat following re-transplantation of the pituitary graft under the temporal lobe. Note complete atrophy and leucocytic vaginal content. x 400.

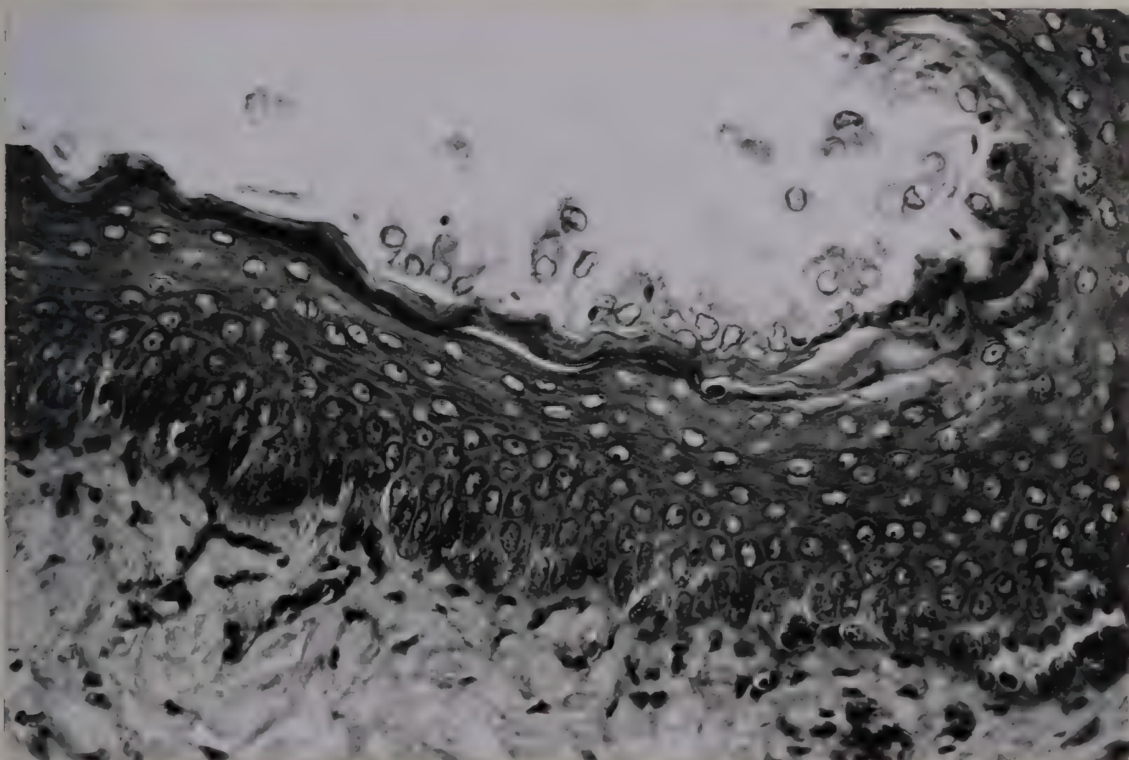

Figure 20. Vaginal mucosa from a rat that had re-cycled following re-transplantation of the pituitary graft under the median eminence. Note maximal repair and the presence of a superficial layer of small epithelial cells characteristic of early proestrus. x 400.

cytology was similar to that in kidney grafts. The cells were uniformly of small size and completely undifferentiated (Figure 21). Each of the grafts that had been well placed under the median eminence showed a significant degree of differentiation whether the animal had cycled or not. Nevertheless, the number of typical anterior lobe cells was far greater in the median eminence grafts from re-cycling animals. In sections of these grafts, large cells can be seen which have the shape of normal basophils, stain purple-blue and possess a distinctly prominent Golgi apparatus situated at a distance from the nucleus. A few of these cells are shown in Figures 22 and 23. Small and large acidophils can also be seen. In addition, some atypical cells can be seen in the median eminence grafts, especially in those belonging to non-cycling animals. These cells have a signet ring appearance, typical of "castration cells" which they are believed to be. These cells are represented in Figure 24.

Pituitary capsules. Histological examination of serial sections of "sellae" showed that in many animals there was a complete absence of hypophyseal remnants. However, in some animals from the non-cycling group and 3 animals from the re-cycling group, very small, undifferentiated fragments were retained. Considering their size and undifferentiated state, it is highly improbable that these fragments were able to exert any effects. Even though a highly differentiated fragment, twice the size of any found in the other animals

[illegible] similar to that in kidney grafts. The cell [illegible] mostly of small size and completely undifferentiated ([illegible]). [illegible] of the grafts that had been well placed under [illegible] showed a significant degree of differentiation [illegible] cells was far greater in the medium [illegible] grafts from [illegible] animal. In sections of [illegible], large cells can be seen which have the shape [illegible] situated at a distance from the lumen. [illegible] of these cells are shown in Figures 22 and 23. Small and large [illegible] can also be seen. In addition, some atypical cells [illegible] appearance, typical of "castration cells" which they are referred to as. These cells are represented in Figure 24.

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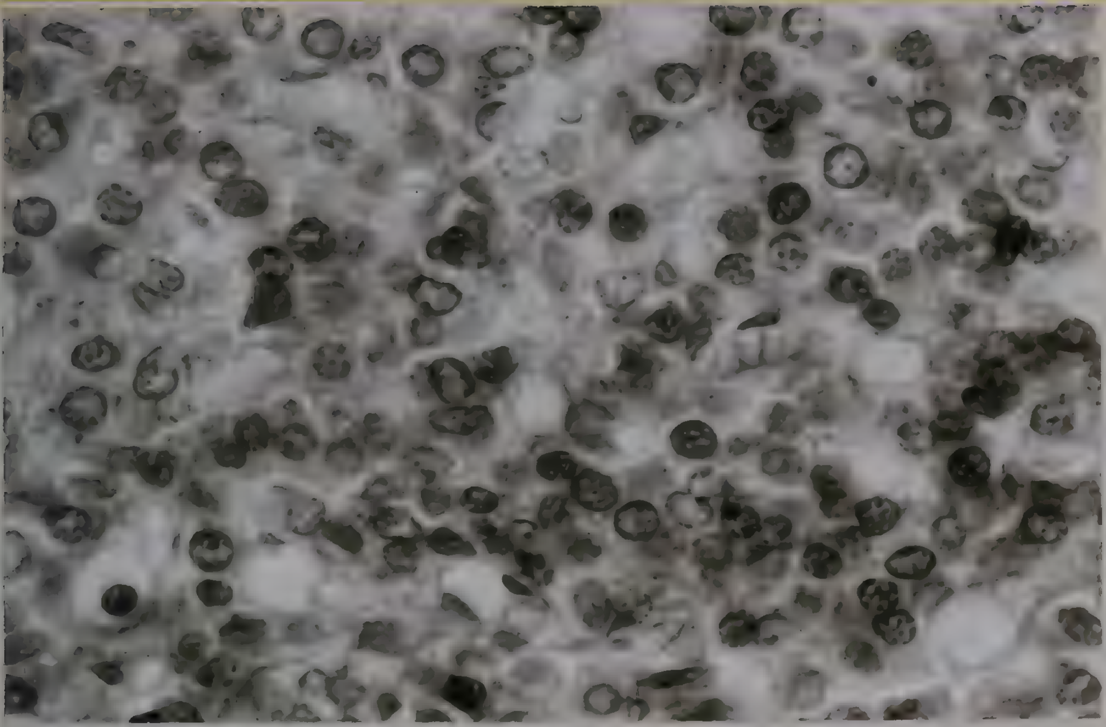

Figure 21. Pituitary graft in the kidney. Note the uniform population of small, relatively undifferentiated cells. x 1000.

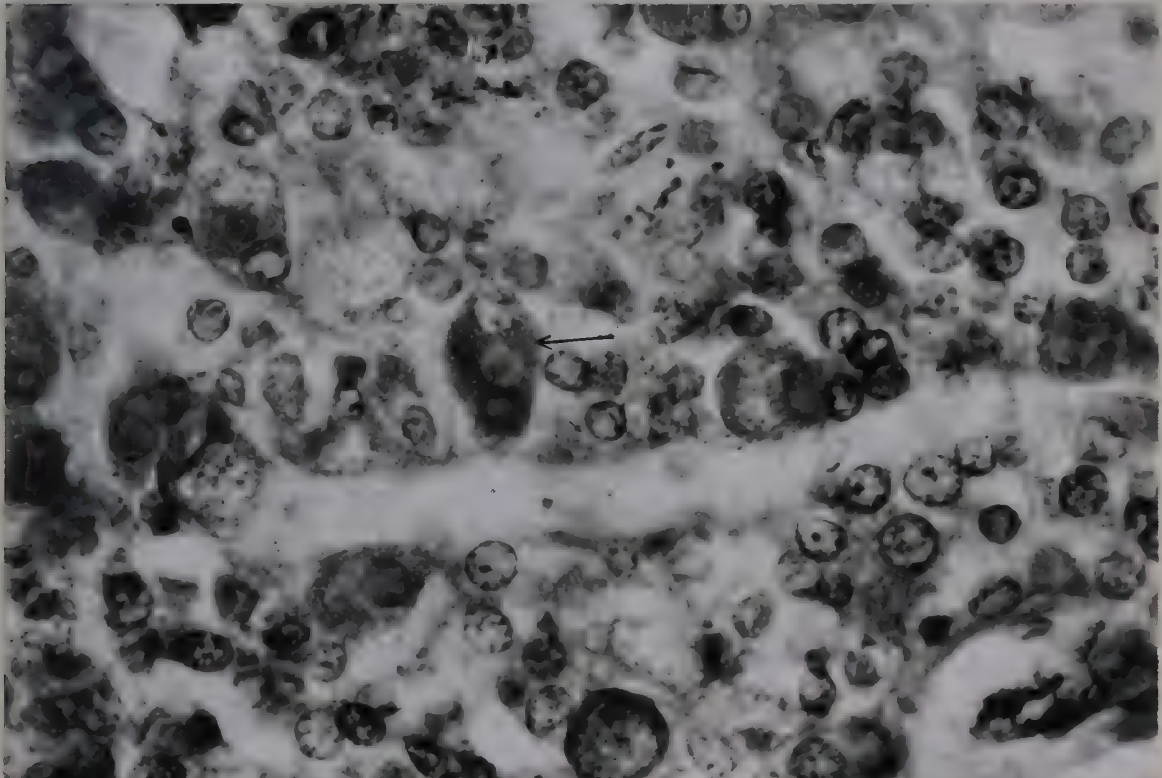

Figure 22. Pituitary graft re-transplanted from the kidney to the median eminence (animal with cycles). Note the presence of large cells. Arrow points to a basophil. Also note the small vacuole above the negative image of the Golgi apparatus. x 1000.

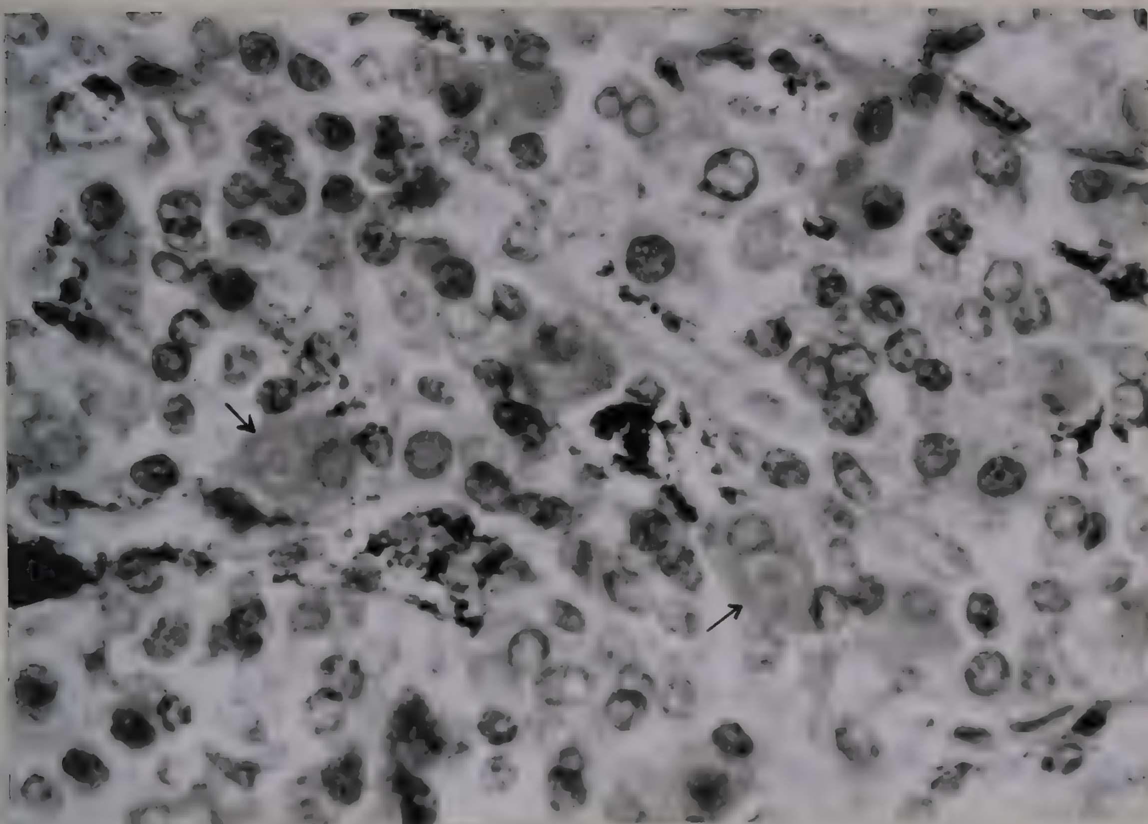

Figure 23. A different field of the same section presented in Figure 22, showing 2 characteristic basophils (arrows). x 1000.

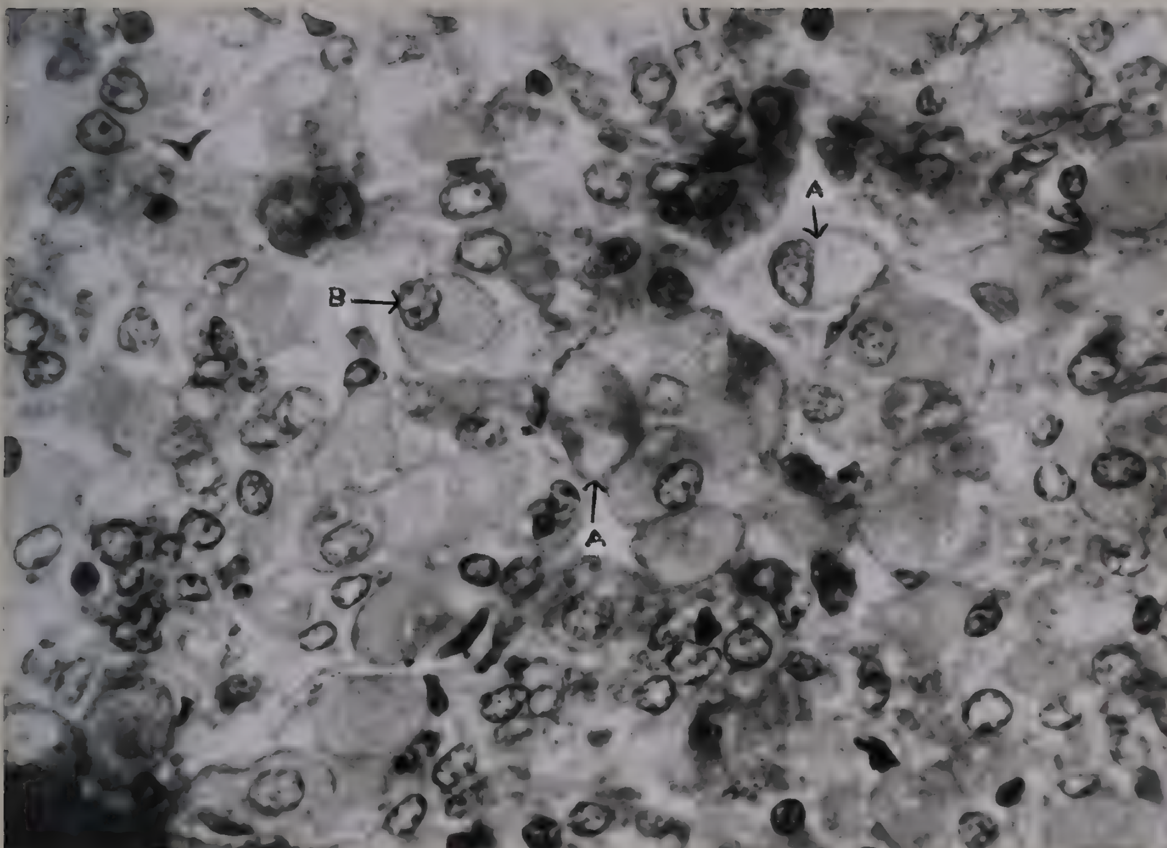

Figure 24. Pituitary graft re-transplanted from the kidney to the median eminence. This graft is the most differentiated of all those taken from animals that did not re-cycle. Although numerous large cells are present, no normal basophils are seen. The only basophils are those with large vacuoles, "castration cells" (A). The other large cells (B) have staining properties of acidophils (red with acid fuchsin). x 1000.

of the series, was present in one non-cycling rat, it was still too small to cause any appreciable difference, as shown by ovarian atrophy as complete as in the other animals.

DISCUSSION

Throughout this study it has been seen that a loss of FSH and LH secretion and the persistence or enhancement of LTH secretion are the general findings whenever the pars distalis is effectively dissociated from the hypothalamus. Whether the dissociation is accomplished by actual transplantation of the gland or by stalk-section with prevention of vascular regeneration, the results are similar. In the grafting experiments this was true regardless of the site used for transplantation: kidney, eye, fascia of the neck (Everett, 1954) or connective tissue outside the kidney capsule. The secretory capacities of the graft were not influenced by the time of the cycle when the pars distalis was transplanted: estrus, diestrus or proestrus. In unpublished experiments Everett found that when the gland was transplanted in the midst of the progravid phase of pregnancy, it continued to maintain the corpora lutea; thus, LTH secretion must have resumed very promptly. Even the pituitaries of new-born rats are capable of secreting LTH when transplanted into a kidney of the hypophysectomized mother (Nikitovitch, unpublished).

Before discussing further the secretion of the various gonadotrophins and the influence of the hypothalamus,

consideration must be given to certain findings encountered in the end organs that serve as indicators of LTH and progesterone secretion, respectively.

It was seen that the presence of reactive corpora lutea was necessary to confirm LTH secretion. If a normal ovary is examined at any time of the cycle, corpora lutea in various degrees of degeneration can always be seen. The factors causing luteolysis have not been elucidated, although Greep (1934) and Greep and coworkers (1942) have suggested that the combination of FSH and LH may serve in this manner. In the present study no attempts were made to identify the substance(s) responsible. However, certain information can be deduced about the time of the cycle when the phenomenon occurs. The beginning of the effect on the current set of corpora lutea is evidently delayed until late diestrus, for the corpora lutea proved to be equally functional whether the pars distalis was transplanted during estrus or the second day of diestrus. On the other hand, it was seen that when the pars distalis was grafted during proestrus the corpora lutea of most animals were incapable of function*. Thus, luteolysis must be well advanced at this

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*The three exceptional animals in the proestrus transplantation group which had functional corpora lutea are assumed to have been not in true proestrus, in spite of the vaginal smear record. This can be expected in a small proportion of rats in this colony at any given time, and true proestrus will be found on the following day (Everett, personal communication).

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[illegible] to confirm HHH production. If a normal [illegible] at any time of the cycle, corpora lutea [illegible] [illegible] not been eliminated, although Crocq (12) [illegible] (14) have suggested that the [illegible] may occur in this manner. In the present study, [illegible] made to identify the substance(s) [illegible] information [illegible] defined as the time of [illegible] phenomenon occurs. The [illegible] of the effect [illegible] not of corpora lutea is evidently delayed [illegible], for the corpora lutea proved to [illegible] equally [illegible] the second day of diestrus. On the other hand, [illegible] when the [illegible] were [illegible] the corpora lutea of [illegible] [illegible]

[illegible] three oestrous animals in the [illegible] functional corpora lutea [illegible] in spite of the [illegible]. In a small proportion of [illegible], and these [illegible] will be found on the [illegible]

stage of the cycle. This is in accord with the report that patches of fatty necrosis appear in corpora lutea during early proestrus (Everett, 1945).

An observation of interest made in the proestrus group of animals was that subsequent ovulation never occurred even when the pars distalis was transplanted very near the time of LH release (Everett, 1956b). Although the grafted gland must contain at that time large amounts of LH, the absorption of this hormone from the transplant can be presumed to be too slow to be effective.

As the indicator that the corpora lutea were actually secreting progesterone, the ability of the uterus to produce deciduomata was utilized. Reactivity of the endometrium to trauma was retained for as long as 74 days following the original operation (transplantation or stalk-sectioning). During normal pseudopregnancy in the intact rat, by contrast, the reactivity is lost after the seventh day (Long and Evans, 1922; Allen, 1931). As judged by the atrophic state of the follicular apparatus and the complete atrophy of the vaginal epithelium, no estrogen secretion is stimulated by a grafted pars distalis or by a stalk-sectioned pars distalis that remains completely separated from the hypothalamus. This indicates that the decidual reaction is not directly dependent on estrogen secretion, contrary to the general belief that only when the uterus has recently been stimulated with estrogen can

progesterone secretion in the normal, or its injection in the castrated rat, induce the formation of deciduomata following traumatization (Weichert, 1928; Verlardo and Hisaw, 1951).

It is a new finding that the existence of an earlier deciduoma does not preclude a second response, at least in the opposite uterus. The animals in these studies were ideally suited to establish this point, which could not have been done in a normal pseudopregnant animal due to the limited duration of reactivity mentioned above. How long a deciduoma can be maintained cannot be answered without future systematic study, although it can be stated from present knowledge that the persistence is not unlimited.

One of the main objectives of the present study was to determine whether the transplants of the pars distalis to the eye compare functionally with those to the kidney capsule. It is now apparent that this is true and that grafts in the eye do indeed secrete LTH in amounts sufficient to support function of corpora lutea. Other investigators (Martin, 1936; Richter and Eckerts, 1937; Westman and Jacobsohn, 1940) did not encounter this simply because they used hypophysectomized animals into which homotransplants were introduced at a later date. Such animals unquestionably lacked corpora lutea that could respond. Although intraocular transplants of pars distalis are now seen to be capable of secreting LTH, it appears that the kidney is a more favorable site than the

eye, at least in rats. The kidney grafts are always larger and very well vascularized, retaining the typical sinusoids found in the gland _in situ_, well injected with erythrocytes. On the other hand, eye transplants are often very small, their vascularization is scanty and the number of erythrocytes seen in the sections, few. The anterior chamber of the rat eye at best offers a very restricted space, and this alone may account for the differences found. Although it has been claimed by some authors, notably Buxton (1936), Martin (1936) and Schweizer and coworkers (1937), that cellular differentiation is retained in intraocular grafts, this was not true in the present study. Both the eye and kidney grafts were made up of small, relatively undifferentiated cells which appear to be acidophils and chromophobes, previously described by Everett (1956a).

A further objective was to compare the effects of stalk-sectioning with those of transplantation. The experiments have demonstrated that, much as when it is transplanted, when the rat pituitary gland is effectively isolated from the hypothalamus by placement of a barrier to vascular regeneration, the secretion of FSH and LH ceases but LTH secretion continues or is enhanced. In both instances the follicular apparatus and interstitial tissue become atrophic and the ovaries have large functional corpora lutea. However, it is noteworthy that the corpora lutea in the stalk-section group were always larger than those in the transplantation series. It is conceivable that more parenchyma

is retained in the pars distalis after stalk-sectioning than after transplantation; this could possibly account for a greater LTH secretion, and this in turn for the larger corpora lutea.

Westman and Jacobsohn (1938b) suggested that pseudopregnancy can be induced by cervical stimulation following stalk-sectioning. The results in the present study demonstrate that stalk-sectioning alone is sufficient to initiate this state. Such a conclusion was reached by Everett (1954) from the indirect evidence that simple transplantation of the pars distalis would invoke the state of pseudopregnancy. The present experiment supports this by direct evidence. Furthermore, it was found that not only stalk-sectioning but even a slight pressure on the brain near the median eminence causes pseudopregnancy. It may be that irritation produced by thus lifting the brain brings about "firing" of neurons in hypothalamic nuclei or causes a slowing of blood flow in the hypophyseal portal vessels. Worthington (1955b, 1957) has reported, from direct observation of the pituitary stalk in the mouse, that blood flow here is reduced when epinephrin is locally applied and in response to peripheral pain. At present little is known of the special physiology of these vessels and their tributaries, but it is conceivable that a greatly modified rate of flow, if long continued, could somehow be a cause of pseudopregnancy.

For the present, we are less concerned with the actual

initiation of pseudopregnancy than with its continuation once begun. Whether or not it will persist after the stalk is cut depends on how well the barrier is placed. It was seen that when regeneration of hypophyseal portal vessels was permitted, pseudopregnancy was interrupted by the onset of cycles. Otherwise it persisted throughout the experimental period, as in the transplantation experiments. Although the decidual reaction was not always elicited in the group of animals with the defectively placed barrier, it is believed that these animals were nevertheless in true pseudopregnancy, as judged by the presence of large corpora lutea. The lack of deciduomata in most of this group is believed to be due entirely to the fact that uterine trauma was performed too late to be effective. As mentioned earlier, during normal pseudopregnancy no decidual reaction is obtained when the uterus is traumatized after the seventh day.

The final objective was to re-transplant autografts of the pars distalis to a site close to the median eminence, after the gland had become established in the kidney. In such a re-transplantation there would be a possibility of re-establishment of vascular connections much as in the case of stalk-section without effective barrier. The circumstances which distinguish re-transplantation from stalk-sectioning, or from direct transplantation of a fresh gland to the median eminence region immediately after hypophysectomy, are: (1) that the gland is

twice insulted, with resultant loss of tissue, and (2) that while in the kidney the gland becomes de-differentiated cytologically and functionally. It has now been shown that such a gland when placed in proximity to the hypothalamus, can nevertheless regain its ability to secrete FSH and LH, as demonstrated by the renewal of estrous cycles and even, in some cases, by pregnancies. The functional differentiation probably occurs subsequent to cytological changes, as discussed later. These findings show conclusively that the operative damage and adverse effects of transplantation per se are not responsible for the cytological changes and loss of FSH-LH secretion. Rather, they point to the fact that the separation of the grafts from the hypothalamus causes the observed changes. These observations, furthermore, provide additional proof for the existence of humoral substance(s) that serve as mediators, through which hypothalamic activity is translated to the pars distalis by means of the hypophyseal portal vessels. The existence of such humoral factor(s) in the hypothalamo-hypophyseal linkage has been suggested by several authors and the whole subject has been recently reviewed by Harris (1955) and Benoit and Assenmacher (1955). That the results observed can be accounted for by a direct innervation of the graft is a very improbable explanation since it has been shown that nerve fibers of the hypothalamo-hypophyseal tract do not regenerate (Fisher et al., 1938).

It has been mentioned above that functional differentiation

follows cytological changes. This statement is based on the fact that cellular differentiation could be seen in all glands which were placed under the median eminence, whether the animals had cycled or not. The finding that "castration cells" were especially numerous in the non-cycling animals is not difficult to explain, considering the completely atrophic state of the ovaries in these animals. A question arises whether some of these additional grafts would have proven to be functional had the ovaries been built-up by FSH-LH injections. It may be that some ovaries were too atrophied to respond to a moderate FSH-LH secretion which might actually have existed in some cases.

It is quite remarkable that the graft, which is composed of relatively undifferentiated cells while in the kidney, has enough plasticity to regain semi-normal structure and function. Except for some difference in staining properties, many normal-appearing basophils and acidophils are found in the grafts of the animals that re-cycled. The question arises whether these cells were all present in the kidney graft in a dormant state or whether, after re-transplantation, they formed *de novo* from undifferentiated reserve cells. Only further study can answer this by following, with appropriate cytological techniques, the changes within the grafted tissue with the progress of time after transplantation or after re-transplantation.

SUMMARY AND CONCLUSIONS

The study was based on the observation by Everett that in the rat the pars distalis of the hypophysis, when transplanted to the kidney on the day of estrus, secretes adequate amounts of LTH while it loses its ability to secrete FSH or LH. A variety of experimental conditions were imposed to determine whether the pars distalis will still behave similarly when transplanted to the anterior chamber of the eye of an estrous rat, or when transplanted to the kidney on different days of the cycle, or when subjected to stalk-section *in situ*. It was found that the pars distalis when grafted to the anterior chamber can secrete LTH, as indirectly measured through the function of corpora lutea and the resulting ability of uteri to respond to trauma with a decidual reaction.

When the pars distalis was autografted to the kidney on various days of the cycle it was found that LTH is secreted in all cases. This was not at first recognizable when the transplantation was performed in proestrus. Luteolysis occurs on that day of the cycle and for this reason no responsive corpora lutea, which are necessary for detection of LTH, are present at the time of transplantation. When new corpora lutea were produced by hormonal treatment, it was shown that the glands transplanted during proestrus also secrete LTH.

With the stalk-section technique it was demonstrated that the pars distalis, when completely separated from the hypothalamus by a barrier, continuously secretes LTH, as shown by unlimited persistence of pseudopregnancy. The pseudopregnancy could be initiated by simple stalk-section or even by slight mechanical injury to the hypothalamic region itself, but it ends rapidly unless the regeneration of the hypothalamo-hypophyseal connections is prevented.

The possibility was investigated that the injury inflicted to the pars distalis by transplantation may be the cause for the de-differentiation of the kidney graft and the loss of FSH and LH secretion which follows. It was found that kidney grafts upon re-transplantation under the median eminence of the tuber cinereum are capable of re-acquiring a "normal" gonadotrophin function and differentiated cytology. Especially notable is the return of the basophils and castration cells, which are never found in the grafts in the kidney. Control animals, in which the grafts were moved from the kidney to a location under the temporal lobe of the brain, failed to regain the capacity for FSH-LH secretion or to undergo cytological re-differentiation. Recovery of these attributes in the grafts near the median eminence took place in spite of the additional injury brought about by the second transplantation.

In general, it may be concluded from these present studies, that whenever the pars distalis is removed from the influence of the hypothalamus, whether by stalk-section or transplantation,

it loses its ability to secrete FSH and LH while it always continues to secrete LTH regardless of the site or time of transplantation. From the re-transplantation experiments it can be concluded that the loss of function and of cytological differentiation observed in pituitary grafts is not due to the injury which occurs during transplantation, but rather to the fact that the gland is placed at a distance from the hypothalamus. Thus, it cannot be reached by humoral substance(s) which appear to be of paramount importance for the stimulation of normal pars distalis function and which are usually transmitted to the gland via the hypophyseal portal vessels.

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CURRICULUM VITAE

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<u>Publications</u>:

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